Annual Red Imported Fire Ant Conference

March 22 - 24 2005 Gulfport, MS

Hosted by:

USDA, APHIS, PPQ, CPHST

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Acknowledgments

On behalf of the 2005 Red Imported Fire Ant Conference Arrangements Committee thank you to all of the attendees, presenters, and sponsors that participated in making the conference such an outstanding success. As the committee chairman, I wish to extend personal thanks and admiration to all of the members of the 2005 arrangements committee. Thank you Doug Meloche, our SIPS secretary, for deftly arranging and setting-up the agenda, proceedings, registration, maintaining contact linkages, registration gifts, and the other incalculable number of details that helped keep the conference running. Thank you Shannon James for gathering, arranging, and displaying all the presentations into an excellent, smooth running PowerPoint® slide show. A good show indeed! Thank you Lee McAnally for assembly and maintenance of the conference sponsor placards and posters and for assisting with the slide show. Also, thank you for help in all areas of need throughout the conference. Thank you to Shannon Wade, Jennifer Lamont, and Mary Collins for help throughout the conference. Special thanks are especially due to Anne-Marie Callcott, SIPS Leader and Fire Ant Diva, for moral, time, and resource support. Also, thank you for lending your experience and historical knowledge to the committee during the entire conference process.

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Sincere gratitude and regards to Dr. Jerome Goddard, State Medical Entomologist, Bureau of Environmental Health, Mississippi Department of Health, for his informative and very entertaining presentation, "Health Effects of Human-Fire Ant Interactions."

Many thanks to all of the conference sponsors and supporters. Your dedication and continued support are what made this annual conference financially possible.

Sincerely,

Ron Weeks
2005 RIFA Conference Chairman

Proceedings of the

Annual Red Imported Fire Ant Conference
March 22 - 24, 2005

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The 2006 Red Imported Fire Ant Conference will be held in Mobile, AL at the Radisson Admiral Semmes Hotel on March 28th thru 30th, 2006.

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On behalf of the USDA, APHIS, PPQ, Soil Inhabiting Pests Section, I would like to welcome all of you to the Mississippi Gulf Coast. I hope while you are here you will have the opportunity to experience our beaches, casinos, and local restaurants. Please note that we have other less obvious attractions: our art museums (George Ohr – potter; and Walter Anderson – artist and sculptor), and our historical homes such Beauvoir, Jefferson Davis's last home (president of the Confederate States of America).

Since we have many newcomers every year to this meeting, I thought I would take this opportunity to briefly summarize how this annual meeting started and where we are now. The annual Imported Fire Ant Conference began over 30 years ago as a small group of scientists who wanted to meet once a year to compare notes and exchange ideas. This informal group meeting has grown from the original 15-20 scientists to more than 125 scientists and students from various research, regulatory and educational organizations, as well as industry and other interested parties. Originally we were all from the southeastern United States, and in later years USDA-ARS cooperators from the fire ants' homelands in South America joined us. Then about 10 years ago the imported fire ant invaded the western areas of the U.S., bringing New Mexico and California into our group. About 5 years ago, Australia was invaded by the imported fire ant, and the Australians have become regular attendees at our annual meeting. This year we would like to welcome the latest international areas that have been impacted by the imported fire ant. The imported fire ant has made its way to Taiwan, Hong Kong and mainland China, and representatives from these areas are joining us at our meeting this year and we welcome them. We will have a question and answer period tomorrow afternoon for all our international guests, but I encourage all of you to interact with our international visitors prior to that session and offer your expertise.

In spite of the fact that the imported fire ant community is growing and expanding outside the borders of the United States, we are still a very small community and we need to continue to nurture this meeting to share our ideas, our successes and our failures. I don't think the intent of this meeting has changed much since its inception, it has just become a bit larger and the players change a bit more often than in years past. While some of us are interested in eradication and others in management or quarantine, it is important that we maintain our connections to all aspects of the imported fire ant problem and continue to share information through this meeting. I believe the program organizers have allowed for substantial time for interactions outside the presentations.

In closing, I anticipate an interesting and productive meeting based on the agenda. I hope you enjoy your time here in south Mississippi and if you need anything during your stay, please see any of us for assistance.

FIRE ANT VENOM ALKALOIDS: UPDATE ON MAMMALIAN TOXICITIES AND CLINICAL RELEVANCE

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Recent results from our laboratory have documented that the alkaloidal compounds that are the majority components of red imported fire ant (*Solenopsis invicta*) venom are capable of producing cardiovascular and central nervous system toxicity in rodents. These data provide a rational basis for interpretation of adverse clinical sequelae following massive fire ant stinging attacks in elderly, debilitated patients and in infants. The present investigations examine additional aspects of systemic mammalian toxicity following venom alkaloid administration. Responses to intravenous and intraperitoneal administration of the structurally verified, synthetic *S. invicta* venom alkaloid, solenopsin A (*trans*-2-methyl-6-n-undecylpiperidine) were evaluated in anesthetized, artificially ventilated Sprague-Dawley rats and in conscious, freely moving ICR mice. In addition, a battery of behavioral indices were evaluated in mice following intraperitoneal administration of isosolenopsin A (*cis*-2-methyl-6-n-undecylpiperidine). Neither combined pretreatment with histamine-1 and histamine-2 receptor antagonists, cimetidine (10 mg/kg) and pyrilamine (2.5 mg/kg) nor with the mast cell stabilizer, cromolyn sodium (25 mg/kg) significantly altered the marked negative inotropic, chronotropic or hypotensive responses to i.v. injection of solenopsin A (15 mg/kg), indicating that mast cell degranulation and histamine release do not mediate these actions. Further studies in mice indicated that neither solenopsin A nor isosolenopsin A exerted exerted behavioral activity other than a mild ataxia in doses up to 30 mg/kg, i.p. A review of media across the Southeastern United States revealed an additional 10 cases of massive human stinging attacks with generally poor outcomes. The results continue to indicate that *S. invicta* venom alkaloids possess meaningful mammalian toxicities and may contribute to adverse cardiovascular effects noted in susceptible humans after massive fire ant stings.

Introduction

The venom of the red imported fire ant, *Solenopsis invicta*, contains up to 95% by weight of disubstituted piperidine alkaloids, termed solenopsins [MacConnell et al., 1971], isosolenopsins [Jefford and Wang, 1993], or dehydrosolenopsins [MacConnell et al., 1971], depending upon the enantiomeric configuration and alkyl or alkenyl carbon chain in any individual compound. At least five compounds, three (2R,6R)-*trans*-2-methyl-6-alkylpiperidines, termed solenopsins, and two (2R,6R)-unsaturated *trans*-2-methyl-6-alkenylpiperidines, the dehydrosolenopsins are found in varying concentrations in native venom. Alkyl chains consist of 11 carbons (C11, solenopsin A), 13 carbons (C13, solenopsin B) and 15 carbons (C15; solenopsin C). Dehydrosolenopsin B is a C13 compound with a double bond between C4 and C5 of the chain, while dehydrosolenopsin C is a C15 compound with the double bond between C6 and C7. Smaller amounts of the (2R,6S)-*cis*-isomers (referred to by the prefix, iso-; eg., isosolenopsin A) of several of these alkaloids are also present in fire ant venom [Brand et al., 1972; LeClerq et al., 1994]. The present experimental protocols utilized the racemic forms of two synthetic venom alkaloids, *cis*- and *trans*-2-methyl-6-*n*-undecylpiperidine. Both of the isomers, (2R,6S)-*cis* (isosolenopsin A) and (2R,6R)-*trans* (solenopsin A), are found naturally in *S. invicta* venom [Brand et al., 1972; LeClerq et al., 1994; Rhodes, 1977; LeClerq et al., 1996]. We have recently reported that both solenopsin A and isosolenopsin A cause negative inotropic, chronotropic and hypotensive responses in anesthetized and artificially ventilated rats, while acute respiratory failure and seizures are noted to solenopsin A in conscious rats upon i.v. injection [Howell et al., 2005]

A total of 10 patient encounters resulting from massive fire ant sting attacks have been documented in the medical literature [deShazo et al., 2004]. Among those were elderly individuals with preexisting cardiopulmonary disease who have experienced cardiorespiratory failure and death within a week of the attacks [Amador and Busse, 1998; DeShazo and Banks, 1994; deShazo and Williams, 1995; DeShazo et al., 1999; Diaz et al., 1989]. We have suggested that the high mortality rate in these individuals may reflect a component of direct toxicity from fire ant venom alkaloids [deShazo et al., 2004]. Therefore, additional information is needed on the putative toxic properties of the alkaloid components of *S. invicta* venom.

Methods

Male (275-325 g) Sprague Dawley rats and male ICR mice (25-30 g) were purchased from Harlan Sprague Dawley. All animal use protocols were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Racemic (±) solenopsin A hydrochloride and racemic (±) isosolenopsin A hydrochloride were synthesized by previously reported methods [Howell et al., 2005; Nagasaka et al., 1989] and have physical (mol. wt. 289.5) and spectral data identical to those reported by others.

Rats were anesthetized with isoflurane and indwelling femoral arterial and venous catheters, and a tracheal ventilatory cannula implanted. A left ventricular catheter was inserted via a carotid artery. Drugs or 5% cyclodextrin vehicle in saline were injected i.v. in gallamine-paralyzed, ventilated rats. Maximal changes in heart rate (HR), mean arterial pressure (MAP), and dP/dt were recorded. Pretreatment with either histamine receptor antagonists, cimetidine hydrochloride (10 mg/kg, i.v.) and pyrilamine hydrochloride (2.5 mg/kg. i.v.) or the mast cell stabilizer, chromolyn sodium (25 mg/kg, i.v) was given 15 min. prior to injection of (±) solenopsin A (15 mg/kg; i.v.). Data are expressed as maximal percent change from control values measured immediately before solution administration.

Male ICR mice (25-35 g) were housed individually and pretreated with i.p. injection of 5% cyclodextrin vehicle, MK-801 (0.5 mg/kg) or solenopsin A (15 or 30 mg/kg). A second injection of 150 mg/kg, i.p., was given to each mouse 15-20 min. thereafter. The incidences of NMDA-induced convulsions and lethality were recorded for the ensuing 30 min. period. Data are expressed as % incidence for each end-point.

Male ICR mice (25-35 g) were housed individually in a partially darkened, secluded area for a minimum of one hour prior to testing. Each mouse was given a single i.p. injection of 5% cyclodextrin vehicle or isosolenopsin A (3, 10 or 30 mg/kg). Beginning 30 min. following injection, each mouse was evaluated according to the Irwin battery of behavioral indices [Irwin, 1968] over the ensuing one hour period. Data are expressed as Irwin battery scores.

Manual searches of all available on-line archives of U.S. newspapers in areas known to have the highest levels of fire ant infestation were conducted for reports of massive fire ant stinging attacks. These included searches from 1989 until 2004 of 42 newspapers in Texas, 34 in Florida, 26 in Georgia, 15 in Tennessee, 14 in Arizona, 12 in Alabama, 12 in Mississippi, 10 in

Louisiana, 10 in South Carolina and 7 in Arkansas. Care was taken to compare each new case identified to previously reported cases to ensure that no duplication of reporting occurred.

Results

Mast Cell Degranulation does not Mediate Cardiovascular Depressant Actions of Solenopsin A

Intravenous administration of solenopsin A (15 mg/kg) resulted in significant bradycardia, a negative inotropic effect and hypotension in the anesthetized, paralyzed rat (Figure 1). The maximal effects elicited by the alkaloid were achieved within 15 to 30 minutes of administration. It has previously been shown that administration of 5% cyclodextrin, (volume-matched control) caused mild (5-10% increase) in heart rate, blood pressure and left ventricular contractility at the volume utilized [Howell et al., 2005]. In separate groups, neither intravenous pretreatment with combined histamine-1 and histamine-2 antagonists (cimetidine and pyrilamine) nor with the mast cell membrane stabilizer, chromolyn sodium significantly mitigated any of these responses (Figure 1).

(±) Solenopsin A does not Alter Lethality of N-Methyl-D-Aspartic Acid (NMDA) in the Mouse

Administration of NMDA (150 mg/kg, i.p.) to conscious mice caused seizures and lethality in roughly 62% (16/26) vehicle-pretreated mice. The i.p. injection of solenopsin A (15 and 30 mg/kg) had no significant effect on the convulsions or the lethality (Figure 2) induced by NMDA. A mild ataxia and characteristic, "hunched" posture was noted in mice at 30 mg/kg of solenopsin A. In contrast, pretreatment with the NMDA receptor antagonist, MK-801 (0.5 mg/kg, i.p.) induced locomotor excitation and stereotypy in mice, significantly reduced the lethal action of NMDA (Figure 2) and also reduced NMDA-induced convulsions (data not shown).

(±) Isosolenopsin A does not Exert Marked Behavioral Actions in the Mouse

The i.p. injection of isosolenopsin A (3-30 mg/kg) had no pronounced deleterious behavioral actions in mice, as determined by a lack of alteration in the Irwin behavioral battery (Figure 3). In contrast, i.p. injection of 3 and 10, but not 30 mg/kg, of isosolenopsin A significantly reduced the time to first fall, but not the total time during which mice were able to maintain balance on a rotating rod, indicating a mild impairment of locomotor function by low doses of the alkaloid (Figure 4). The impairment was comparable to that induced by the benzodiazepine, chlordiazepoxide (20 mg/kg, i.p.).

Media Reports of Massive Fire Ant Sting Attacks

An additional 10 cases of fire ant sting attacks indoors were identified in media reports between 1991 and 2004 that have not been previously reported in the medical literature. There were 4 attacks in Alabama, 4 in Florida, and one each in Texas and Arizona. One of the attacks occurred in 1991, one in 1994, one in 2000, one in 2001, three in 2002, and three in 2003. While a majority of attacks occurred in long term health care facilities, two attacks were reported to have occurred in hospitals. One half of the newly reported cases involved victims who were immobile on the basis of age or disease or both. Two additional reports of attacks on infants and two previously unreported deaths from fire ant attacks could be identified.

Discussion

The present results provide evidence for systemic, multi-organ toxicity in mammals from the alkaloidal components of *S. invicta* venom. Although the potential for direct toxicity of *S. invicta* venom has been discounted in the past, morbidity and mortality after massive fire ant stings in the elderly and earlier, preclinical data from this laboratory suggested a need for further investigation of *S. invicta* venom toxicity [Howell et al., 2005; deShazo et al., 2004].

Lethal effects of fire ant envenomation are documented in fish, birds and small mammals [Flickinger, 1989; Green and Hutchins, 1960; Travis, 1938]. No basis for these actions has yet been clearly elucidated in the laboratory. However, the data presented here verify previous results showing the induction of bradycardia, hypotension, and negative inotropic effects following intravenous injection of pure synthetic alkaloid components of fire ant venom in the anesthetized rat. The demonstrated ability of venom alkaloids to release histamine *in vivo* [Read et al., 1978; Lind, 1982] suggested histamine as a putative mediator of the cardiovascular responses to administration of the synthetic alkaloids. Nevertheless, blockade of both histamine 1 and histamine-2 receptors had not effect on the cardiovascular depressant actions of solenopsin

A. Inhibition of mast cell degranulation by chromolyn sodium also failed to mitigate such depressant effects, further ruling out involvement of mast cells in these actions.

The seizure activity previously noted in conscious, spontaneously breathing rats following intravenous injection of solenopsin A demonstrated a potential for neurological systemic toxicity of compounds in this series. However, solenopsin A neither enhanced nor inhibited the convulsive activity of the excitotoxin, NMDA, and exerted only modest behavioral inhibitory actions in conscious mice. Similarly, isosolenopsin A caused only minor locomotor dysfunction and no other significant behavioral toxicities in mice at doses up to 30 mg/kg, i.p.

In humans, one individual has been reported to recover from an estimated 10,000 stings without systemic ill effects [Diaz et al., 1989] and a few relatively healthy elderly individuals have experienced massive sting attacks without obvious sequelae. However, four of six chronically ill elderly individuals receiving massive fire ant stings while in nursing homes have been reported to have died in less than one week of the attack [deShazo et al., 2004]. A total of 20 lethal, non-anaphylactic attacks have now been reported in the elderly or infants and, of these, 14 of 20 occurred in health care facilities. Morbidity in the newspaper reports ranged from nightmares to death, though outcomes were not always specified. A total 6 of the these 20 persons known to have experienced fire ant sting attacks died within one week of the incidents. Two new reports of attacks involving infants were found, raising the number to 4 out of 20 known attacks. All infants had serious sequelae ranging from corneal injury to death in one. Infants appear less sensitive than elderly adults, with a lower mortality rate (25%) than adults in similar scenarios (37.5%). Severe medical consequences, including lethality, occurred in only 30% of the 10 patients reported in the public media [Associated Press, 1991; Krueger, 2000; Chachere, 2000a; Associated Press, 2000a; Associated Press, 2000b; Associated Press, 2000c; Associated Press, 2001a; Associated Press, 2001b; Associated Press, 2001c; Carroll, 2003]. In contrast, only 20% of the sting episodes previously reported were associated with no medical consequences [Diaz et al., 1989; deShazo and Banks, 1994]. This may relate to suppression of media exposure associated with litigation that accompanied those attacks resulting in greater morbidity or in lethality.

The present results support the hypothesis that fire ant venom alkaloids can produce adverse cardiovascular toxicities in mammals. These effects may explain some of the reported clinical sequelae of massive fire ant stings. The increasing spread of the red imported fire ant in the continental United States, Puerto Rico, the Virgin Islands, Australia [Solley et al., 2002] and into Taiwan will require further evaluation of toxic properties of *S. invicta* venom alkaloids.

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Case	Age	Sex	Health Status	Location	Clinical Response	Reference
1	Newborn	M	Normal	Private apartment	Scarring, permanent brain damage	1
2+	77 yrs.	F	Unknown	Hospital ICU	Nightmares	2,3,4
3+	87 yrs.	F	Heart disease, Dementia	Nursing home	Death within 1 day	5-12
4	83 yrs.	M	Stroke, Dementia	Nursing home	None Reported	13
5	79 yrs.	F	Not reported	Assisted living facility	None Reported	14
6+	91 yrs.	F	Fractured hip	Nursing Home	None reported	15,16
7	83 yrs.	M	Stroke, fractured hip	Hospital	None reported	17
8	3 mo.	F	Normal	Private home	Death	18
9	36 yrs.	F	Rhett Syndrome	Residential care facility	None reported	19
10	Adult	F	Not reported	Hospital ICU	None reported	19

Table 1. Newspaper reports of indoor fire ant stings. +Cases reported in more than a single newspaper. 1, Associated Press, 1991; 2, Kidwell, 1994; 3, Jackson, 1994; 4, Associated Press, 1994; 5, Krueger, 2000; 6, Chachere, 2000a; 7, Associated Press, 2000a; 8, Associated Press, 2000b; 9, Associated Press, 2001a; 10, Associated Press, 2001b; 11, Associated Press, 2001c; 12, Chachere, 2000b; 13, Isger, 2001; 14, Associated Press, 2002a; 15, Associated Press, 2002b, 16, Associated Press, 2002c; 17, Mearns, 2002; 18, Carroll, 2003; 19, Curet, 2003.

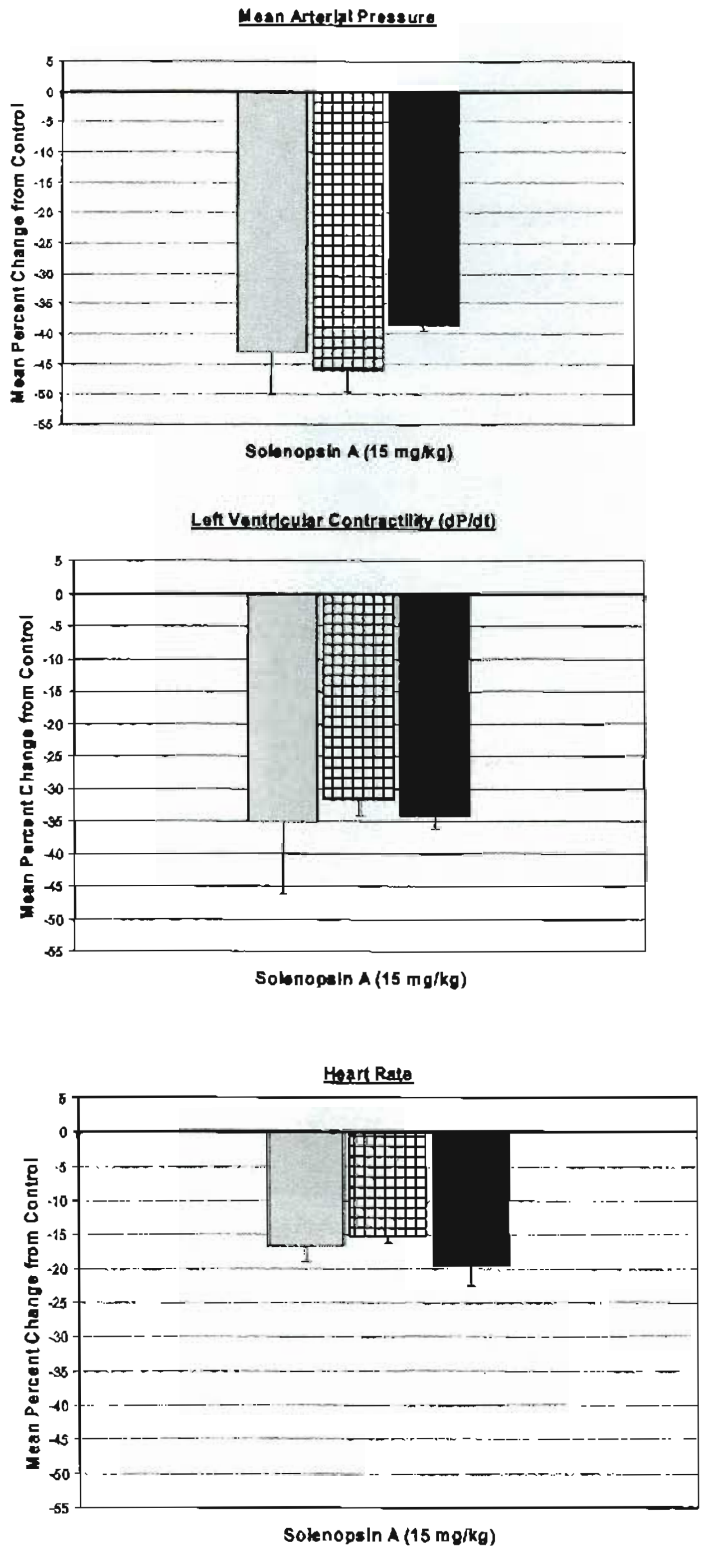
Figure Legends

Figure 1: Effects of solenopsin A (15 mg/kg, i.v.) on percent change from pre-injection control in mean arterial blood pressure (top panel), left ventricular contractility (middle panel), and heart rate (bottom panel) in isoflurane-anesthetized, gallamine-paralyzed, artificially ventilated rats. The first bar (grey) in each panel depicts the response in rats receiving pretreatment with histamine-1 and histamine-2 receptor blockade (n=2), the second bar (checkerboard grid) reflects responses in rats pretreated with the mast cell membrane stabilizer, chromolyn sodium (n=3) and the last bar (solid) represents responses in rats pretreated with 5% cyclodextrin vehicle alone (n=4). Values are represented as mean ± SEM percent change from a control value taken immediately before injection of either vehicle or alkaloid.

Figure 2: Percent lethality in **male mice** receiving the excitotoxin, N-methyl-D-aspartic acid (NMDA; 150 mg/kg, i.p.) 15-20 minutes following pretreatment with 5% cyclodextrin vehicle (left bar), the NMDA receptor antagonist MK-801 (0.5 mg/kg, i.p.; second from left), or solenopsin A (15 mg/kg, i.p.; third from left) and solenopsin A (30 mg/kg, i.p.; far right bar). Numbers in parentheses indicate the number of mice dying within 30 minutes of NMDA injection over the number of mice injected.

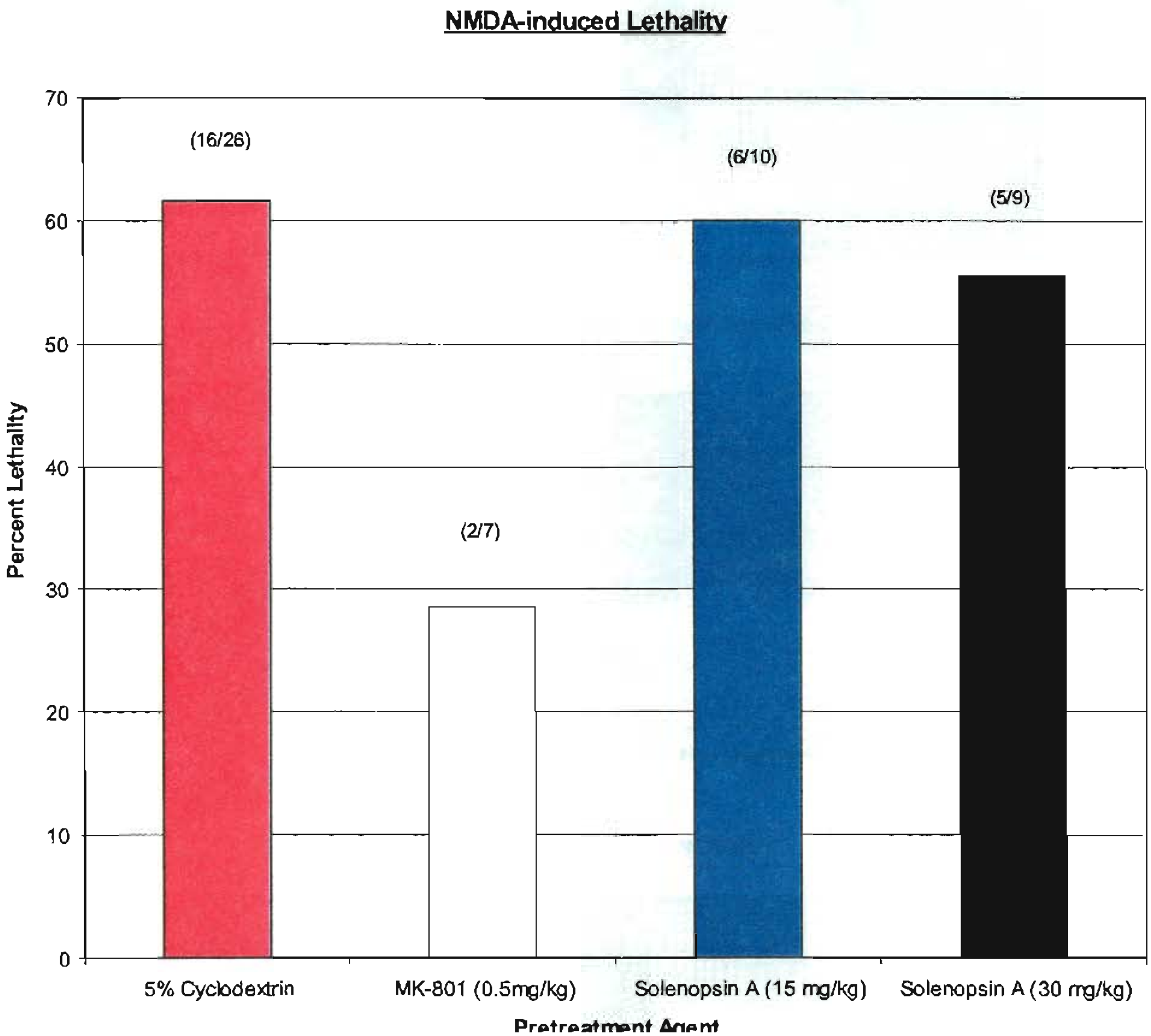
Figure 3: Irwin battery behavioral scores in **male** mice treated with 5% cyclodextrin vehicle or isosolenopsin A (3, 10 and 30 mg/kg, i.p.). No significant differences were noted among groups in the scores.

Figure 4: Top panel: Time to first fall (seconds) in male mice placed on a rotating rod. Bottom panel: Total number of falls of mice placed on a rotating rod during an experimental period. Fifteen mice were in each group. The left-most bar in each panel shows results from mice treated with 5% cyclodextrin vehicle; the second from left, chlordiazepoxide (20 mg/kg, i.p.); the third from left, isosolenopsin A (Iso A, 3 mg/kg, i.p.); fourth from left, isosolenopsin A (Iso A, 10 mg/kg, i.p.) and the right-most bar, isosolenopsin A (Iso A, 30 mg/kg, i.p.). Mean values ± one S.E.M. are depicted. * = statistically different from cyclodextrin control at $p<0.05$.

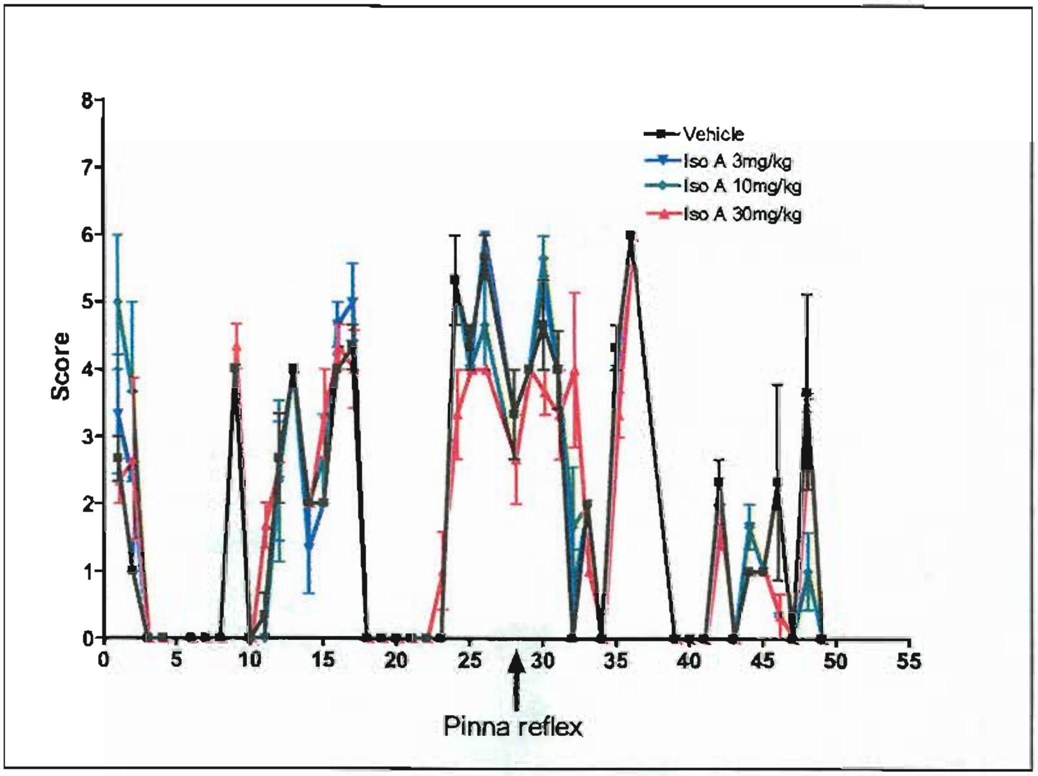


Mean Arterial Pressure
Mean Percent Change from Control
5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
Solenopsin A (15 mg/kg)
Left Ventricular Contractility (dP/dt)
Mean Percent Change from Control
5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
Solenopsin A (15 mg/kg)
Heart Rate
Mean Percent Change from Control
5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
Solenopsin A (15 mg/kg)

Figure 1



NMDA-induced Lethality
70
60
50
40
30
20
10
0
Percent Lethality
(16/26)
(2/7)
(6/10)
(5/9)
5% Cyclodextrin
MK-801 (0.5mg/kg)
Solenopsin A (15 mg/kg)
Solenopsin A (30 mg/kg)
Pretreatment Agent



Vehicle
Iso A 3mg/kg
Iso A 10mg/kg
Iso A 30mg/kg
Score
8
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
35
40
45
50
55
Pinna reflex

Figure 3

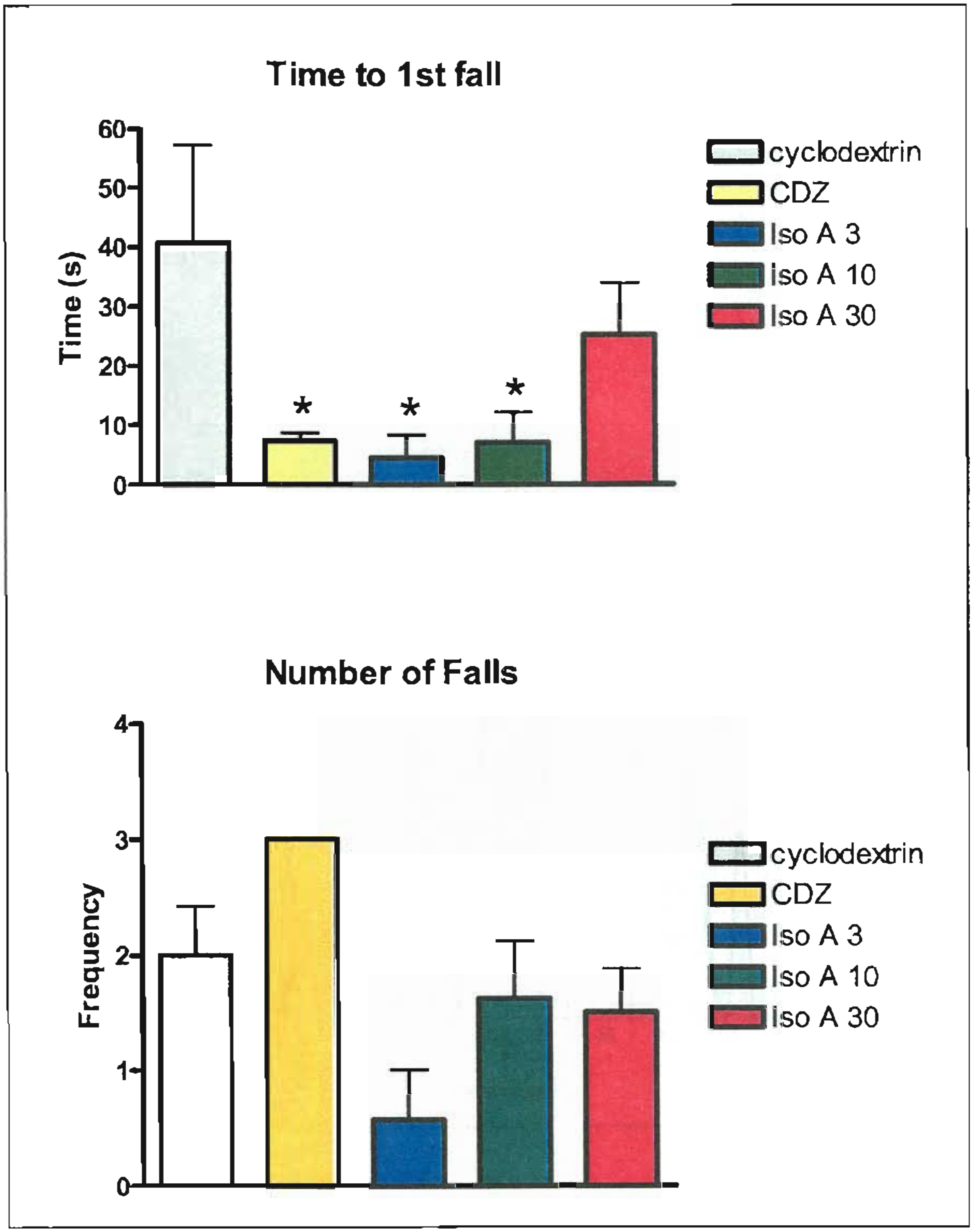

Time to 1st fall
Time (s)
60
50
40
30
20
10
0
*
*
*
cyclodextrin
CDZ
Iso A 3
Iso A 10
Iso A 30
Number of Falls
Frequency
4
3
2
1
0
cyclodextrin
CDZ
Iso A 3
Iso A 10
Iso A 30

Figure 4

Above and Beyond the Community-Wide control efforts in the near-urban areas of Texas

Paul R. Nester, Extension Agent - IPM,
Texas Cooperative Extension,
Houston, TX

Texas Cooperative Extension wants to better understand the awareness of aerial applicators to applying low rates (1 - 2 lb product/acre) of fire ant bait products by air and the capabilities of their aerial equipment to conduct this task. The objective will be to enhance the Texas Cooperative outreach activities to better meet the needs of the aerial applicator.

Until the early 1980's, government organized fire ant control programs were conducted where bait products were exclusively applied by air to fire ant infested areas. Since these programs no longer exist and it has been a decade or longer since general aerial applications of bait products for fire ant control were conducted, the Texas Cooperative Extension wants to survey the existing aerial applicator businesses to ascertain current capabilities.

With the attrition of aviators in this profession and the consolidation of many aerial applicator businesses, it is feared that the experience of applying these low rates may be lost. Plus, the increased security measures around urban areas may place restrictions on their businesses. The ability to fly over urban, near-urban, or highly congested areas has become more difficult, and may be cost prohibitive when considering the aerial application of fire ant bait products to these areas.

Aerial applicator input is needed on these issues to assess their impact on the aerial application of fire ant bait products. Anticipated outcomes of this effort are to develop a list of service providers for Texas counties and post this list on the Texas Fire Ant Project web site (http://fireant.tamu.edu) to help connect those with fire ant control needs to aerial applicators and to create a realistic cost analysis to determine if aerial application should be considered in various situations. A survey has been created and will be sent to all aerial applicators servicing Texas counties in early 2005.

UP-DATE OF RIFARID LLC'S PATENTED METHOD FOR INSTANT ERADICATION OF RIFA COLONIES

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RIFARED LLC introduced its new method for the instant eradication of RIFA colonies at the 2004 RIFA Annual Conference held in Baton Rouge. At that time, the patent application for this new method was pending. This patent application was approved late last year and was awarded on December 14, 2004 as Patent No. US 6,831,104 B2.

The method involves the use of a volatile liquid knockout agent in combination with practically any general-purpose insecticide. We now identify our knockout agent as RIFARID™.

At last year's RIFA Conference, we learned several things that led us to perform additional experiments and tests during the past eleven months. Those of you who attended last year's conference might recall Mr. Ed Hordes' Guest Presentation. Mr. Bordes is the Director of the New Orleans Termite & Mosquito Control Board. In his presentation, he emphasized the need for a treatment method that will instantly eradicate the entire population of RIFA mounds without leaving behind lingering residues of chemical compounds that are possibly harmful to people, to wildlife such as birds, to the good insects that compete with the RIFA and also to those insects that serve as food for our precious bird population. Mr. Bordes told us that the city of New Orleans needs to be able to pick up the phone, call the pesticide company and tell them of the scheduled dates for the Jazz Fest so that they can visit the key outdoor festival sites and effectively eradicate the RIFA mounds just a few days prior to the festival event.

After hearing our RIFARID presentation, Mr. Bordes visited our booth and encouraged us to do further testing to determine if our method can be totally effective using benign insecticides that degrade to harmless compounds in a relatively short period of time. Others visited with us expressing the need for such a treating method and treating formulation for the instant eradication of RIFA mounds infesting such places as playgrounds for children, soccer, softball, baseball, football playing fields and practice grounds. The list is indeed lengthy for those situations where RIFA mounds can be instantly eradicated while leaving the area safe for normal use shortly after having applied the treatment solution.

Since last year's RIFA Conference, RIFARID LLC has been busy responding to what was learned at that conference. During the latter half of last year we were invited to participate in a significant testing program conducted in Montgomery County, Texas under the direction of Dr. Paul Nester and Dr. Bart Drees of the Texas Cooperative Extension Service. RIFARID LLC participated in these tests for the primary purpose of confirming that RIFARID™ will knock out the entire population of a RIFA mound, within a matter of seconds, when the mound is drenched properly. A secondary purpose for participating in the tests was to allow Dr. Nester and Dr. Drees to observe first hand my treatment technique and to instruct me as to how it might be improved.

During these tests, a total of 160 active RIFA mounds were drenched with formulations containing the RIFARID™ KNOCKOUT AGENT. In all cases, the ants upon being contacted with the RIFARID™ were promptly knocked out and rendered motionless within the confines of the mound structure.

For the first 80 mounds that were treated, the surface of the mound was initially wetted with a shower of treatment solution poured from a 2-gallon sprinkler can. Then several holes were punctured into the mound using a slender steel shaft. The remainder of the treating solution was then showered over the mound and into the punctured holes. All treating applications were one gallon per mound regardless of the size of the mound.

After treating the first 80 mounds, Dr. Drees suggested a simple less arduous method for treating the remaining 80 mounds. This method involved removing the sprinkling head from the sprinkler can and pouring onto the mound at a controlled flow rate a single flow stream from a distance of 2 to 3 feet above the surface of the mound The free fall of the liquid flow readily breaks through the crust of the mound thus exposing the many interlocking tunnels and caverns that prevail throughout the mound structure. With an ever widening circular motion, the entire contents of the container can be quickly emptied into the structure of the mound. The knockout agent being highly volatile and much heavier than air quickly vaporizes and permeates the entire mound structure instantly knocking out all of the ants occupying the mound Essentially no ants escape from the basic structure of the mound

With this new treating technique, the last 80 mounds were treated in a much shorter period of time and in a more effective manner. RIFARID LLC now considers this technique as its standard mound treating technique and very much appreciates the advice and guidance from Dr. Drees and Dr. Hester that led to the adoption of this simplified technique.

After completing the tests in Texas. I prepared a table specifying treatment volumes for the various mound sizes. I then performed numerous tests using in combination with the RIFARID™ the most benign of the general purpose insecticides now available to the general public. Most of my tests were conducted with general-purpose insecticides containing esfenvalerate or permethrin insecticides that I routinely use in my garden. In all cases. I achieved an instant 100% kill using the above described treatment technique and the treatment volume appropriate for the size of the mound Without question, RIFARID™ used in combination with benign short-life insecticides can be used to instantly eradicate the entire population of a RIFA mound.

I have demonstrated my present treatment technique to several people who are engaged in the never-ending battle to control heavy RIFA infestations at large municipal recreational facilities. One of these people also has years of experience coping with RIFA infestations at a major golfing facility. This person promptly envisioned using a four-wheeler equipped with a 25 to 50 gallon tank for treating large areas heavily infested with RIFA mounds. His idea involved filling the tank with the proper amount of treating solution, continuously circulating the tank with a battery driven pump and equipping the tank with a specially designed teflon® hose containing an on/off electrically operated valve. He would simply steer the four-wheeler with his right hand and with his left hand apply treatment solution to the RIFA mounds using the specially designed teflon® hose. With this method he envisioned treating an entire recreational park without ever leaving the seat of his four-wheeler.

This is a low manpower approach that can be used for treating any large area heavily infested with RIFA. It provides a more affordable way for treating those large infested areas that are now being ignored

Finally and most important of all the RIFARID LLC approach provides an effective way to instantly kill only the occupants of the RIFA mounds. It does not target insects that are combatants of the RIFA. It does not indiscriminately kill insets that serve as food for our bird population. It does not subject our yards, playgrounds, school grounds and our land in general, to year round coverage with active insecticides.

Fact Sheet ENY-226 published by the Florida Co-Operative Extension Service in Jury of 1994 contained the following paragraph:

> "In areas where native ants and fire ant populations have been reduced or eliminated with insecticides, re-infestation by fire ants may be noticeable within a month after treatment. Fire ants re-infest these areas more rapidly and out-compete other ant species because of their tremendous reproductive capacity and faster colony development. If fire ant control is not maintained the subsequent re-infestation of an area may result in even greater fire ant populations than existed before the application of insecticides."

I grew up just north of Mobile. Alabama. I encountered my first RIFA mound in the early 1940's. I am now 70 years old and have witnessed the alarming spread of the RIFA all the way to California, to Hawaii, to Australia and now to Taiwan and mainland China. I have heard it said time and time again that we can not afford a treatment method that targets individual RIFA mounds; that we must use the broadcasting method that wipes out natural competition to the RIFA. Reading the above paragraph taken from Fact Sheet ENY-226 seems to reveal that what we possibly have been doing in our battle with the RIFA is to eliminate competition by too widely and too indiscriminately employing the chemical broadcasting method. If the insects that compete with the RIFA are totally wiped out, then the battle boils down to only man against the RIFA with man primarily using chemical warfare. Man needs to do those things that will enhance the competitive position of the ant species that compete with the RIFA. We must urgently and drastically increase our attack on the individual RIFA mounds and back off somewhat on the indiscriminate broadcasting of long lasting insecticides. The patented RIFARID LLC treatment method, in my opinion, is the best approach to take in this endeavor.

One word of caution for those in the Pacific realm who are now experiencing RIFA infestations; do not overlook the importance of aggressively and immediately eradicating the individual RIFA mounds as they first appear. As the founder and president of RIFARID LLC, I have new pending patents that teach the use of newly discovered knockout agents that are highly effective in my RIFARID™ formulations. These newly discovered agents are abundantly available in the Pacific realm and can be promptly put to use to instantly and completely eradicate RIFA mounds when they are first discovered.

If anyone hearing this presentation is interested in talking further with me about my recently issued patent or about my newly discovered highly effective knockout agents, please get in touch with me sometime during the remainder of this conference.

Utility Boxes and Pasturelands:
Imported Fire Ant Management Product Assessments for Arinix® and Esteem®

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Integrated pest management (IPM) options for imported fire ants, *Solenopsis* species (Hymenoptera: Formicidae) vary with site and goals (see publications and fact sheets on the Texas Fire Ant Project web site, http://fireant.tamu.edu). They can vary from protecting a small object such as a relay switch in an electrical utility unit to suppressing ants in large landscape areas such as cattle production systems. As examples of ongoing applied research, efficacy assessments of two imported fire ant control products are presented. Final reports will be posted on the web site upon release.

Arinix® (Nix of America, nikkonix@ix.netcom.com) is a permethrin-impregnated nylon product. It can be molded into almost any shape and can provide extended residual control of imported fire ants or other arthropods. Previously, this company developed a 9% permethrin impregnated nylon-type part for Nissan automobiles (SG Tube 1725W) to repel spiders from internal combustion engines for an estimated 10 year period. Permethrin-impregnated nylon cylinders were formulated and aged using a heat-treatment process in order to conduct a series of laboratory trials to assess effects on foraging worker ants.

Foraging structure. Rectangular bases were constructed of 3 by 3 cm pine and measuring 38 by 24 cm. Eight 19 mm (3/4 inch) diameter and 39.5 cm tall dowel rods were placed in holes drilled into the base. Each rod had a nail 9.5 cm from the top so that test cylinders could be fixed with the top edge 7 cm from the top of the rod. One foraging structure was placed in each laboratory colony. Nylon cylinders were placed on dowel rod nails. On top of each rod housing a cylinder, a 7 mm (1/4 inch) long, 4 mm diameter "bead" of Jif® Creamy Peanut Butter (The J. M. Smucker Co., Orrville, OH 44667) weighing approximately 0.3 g was applied using a plastic squeeze bottle. After 6 and 24 hours of exposure to foraging ants, the amount of peanut butter remaining (estimated as percent remaining and converted to grams) and number of ants associated with the peanut butter was estimated. Results were analyzed for each trial using analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test at $P \leq 0.05$.

Results. In the trial presented (Trial 5), we used untreated control, and all 7.8% permethrin cylinders of both 0.8 and 1.2 mm thickness aged 0, 1, 3 and 5 years. All premethrin-impregnated cylinders significantly reduced peanut butter after 6 and 24 hrs. Results document that permethrin-impregnated nylon cylinders can significantly reduce foraging ant activity and provide protection of targets (such as peanut butter bait) from ant foraging when applied as a barrier. Contact with treated cylinders is toxic to the ants, thereby reducing foraging activities. As a "controlled release device," when properly installed these elements may be useful for protecting electronic and other devices contained in small housings such as in utility boxes. The capability of the higher concentration formulation (7.8%) to provide protection for 5 years would be a dramatic improvement over currently available products which must be re-applied annually.

Foraging worker ant activity. A plastic tray measuring 27 by 37 cm and 9.5 cm tall was used to make a temperature-controlled foraging tunnel arena that accommodated tubes from six laboratory colonies. Six 165 cm long clear plastic hoses (Clean and Fill No Spill® Aquarium Maintenance System, Python® Products, Inc., Wisconsin), 2 mm thick with an 8 mm inside diameter, were threaded through holes drilled on both short sides of the tray and sealed using silicone glue. On one end, the tube emerging on the outside of the tray was sealed using a cap from a Corning 50 ml Centrifuge Tube (Corning Inc., Corning, NY), with a hole drilled in the center so that the centrifuge tube could be screwed to accommodate a "target bait" food substance such as peanut butter. From the other end, 125 cm of the hose was allowed to dangle into a fire ant laboratory colony onto a stand housing a dowel rod to allow ants to crawl into the hose. The outside of the long end of the hose was painted with fluon to prevent ants from crawling up the outside.

The foraging tunnel arena was filled with tap water maintained at 7 to 8 cm deep so that hoses would be submerged. A NESLAB Refrigerated Circulating Bath (NESLAB Instruments, Inc., Portsmouth, NH) was used to heat and cool water circulating through the tray housing foraging tunnels. Each day, water was heated to 50°C to remove ants from the foraging arena tunnels and ants were brushed out of the cap of the centrifuge tube. Clean centrifuge tubes housing 5 cm-long piece of clear soda straw (Glad® Flexible) in which a 7 mm "bead" of Jif® Creamy Peanut Butter weighing approximately 0.3 g was applied. Temperature was adjusted each day so that the foraging tunnel arena could be maintained at a specified temperature each 6 hour-long trial period (10:00 a.m. to 4:00 p.m., beginning Dec. 1, 2003). Over successive trial periods (days), temperature settings were randomized to eliminate potential effects of laboratory colony age, food saturation and other possible variables associated with conducting trials in a temperature gradient sequence. After 6 hours of exposure to specified foraging tunnel temperature ants, the amount of peanut butter remaining (estimated as percent remaining and converted to grams) and number of ants associated with the peanut butter was estimated. Mean and standard deviation were calculated for data from each temperature trial.

Using a Sony Camcorder, video tapes were taken of foraging ants crawling throug hoses at increasingly higher temperatures beginning at 2°C through 50°C. Using the editor function of I-Mac movie software, speed at which worker ants crawled was documented (cm/min). A linear regression was performed on data using SPSS for Windows (Lead Technologies, Inc. Version 11.5).

Results. The foraging temperature for *S. invicta* ranged from 7° to 48°C (44.6 to 118.4°F), with optimum food consumption occurring from 25° to 35°C (77 to 95°C). Between the temperature extremes of 10° and 49°C, foraging speed varied for individual ants ranged from 0.21 cm/sec (12.6 cm/min or 5.04 in/min) at 10°C to 3.46 cm/sec (207.60 cm/min or 83.04 in/min) at 48°C. The linear regression between speed (dependent variable) and temperature (independent variable) was significant ($R = 0.71$) with an equation for the line being: speed = -019 + 0.06 temp.

For products such as treatments for use in utility housings, protection must be assessed within the range of temperatures that ants will forage. For using bait-formulated insecticides, treatments should be applied when ants are foraging for food. For use of surface temperatures to control ant foraging activities, extreme foraging temperatures could be used as a method of control. Finally, speed of foraging is important to understand when considering how fast ants can explore areas for resources or recruit rapidly, as in the case of hatching birds, new born animals or indigent patients.

Esteem® Fire Ant Bait (pyriproxyfen, Valent U.S.A., http://www.valent.com), also sold as Distance® Ant Bait, is an insect growth regulator (IGR) that produces a slow but extended period of control of imported fire ant mounds. When applied in the spring, maximum control generally occurs in a month or two of application, whereas fall application does not result in maximum control until the following spring, about 6 months later.

Previous field trials by Texas Cooperative Extension (see reports on http://fireant.tamu.edu) documented that the initial period of ant mound reduction could be shortened for the IGR, fenoxycarb, by blending it with a metabolic inhibitor product such as hydramethylnon (e.g., Amdro®Pro, Probait® or others) and applying each at half rate, thereby not increasing the cost of a treatment. This treatment is now registered as a "hopper blend" application of the IGR, methoprene (Extinhuish®), plus hydramethylnon (Amdro®Pro), and as the pre-blended product, Extinguish® Plus. Furthermore, application of an IGR in a "skip swath" pattern, whereby the product is applied at full rate (1.0 to 1.5 lbs./acre) but skipping every other swath, has been shown to result in control equivalent to the full broadcast application. This application method can reduce the chemical cost of treatment and application time roughly in half.

Currently, the only other IGR registered for use in pastureland is Extinguish® (methoprene) ant bait, and Amdro®Pro (hydramethylnon) is registered for use in cattle and hay pastures. Esteem® treatments, including "hopper blend" and skip-swath" applications, were evaluated for registration in pastureland in these trials. The first initiated Oct. 2003 at Lake Granger in Williamson Co., TX, and a second initiated June 2004 near Dobbin in Montgomery Co., TX.

<u>Procedure</u>. In both trials, plots were established for treatments replicated 4 times. Plots were 100 ft. wide and 800 ft. long (roughly 2 acres) at Lake Granger, and 150 by 300 ft. (1 acre) near Dobbin. Imported fire ant mounds were counted in a sub-plot sampling area (30 ft wide and 740 ft long or 0.51 acre in Lake Granger and two 30 ft wide by 260 ft long swaths or 0.358 acre in rear Dobbin) by disturbing suspected nest or mound sites with a stick or shovel and counting them as active if many (dozens) of worker ants were observed to emerge. Pre-treatment ant mound numbers per sub-plot area were arrayed from lowest to highest and blocked in groups of treatment plots forming replicates or treatment blocks. Treatments were assigned randomly to each replicate or block so that only minor numerical differences occurred for average mound per plot values. Treatments included:

Lake Granger

1. Untreated control or check
2. Pyriproxifen - Esteem (0.5% pyriproxyfen) Fire Ant Bait (Valent U.S.A.), 1.25-1.5 lb/acre (five swaths on 20-ft centers or swath widths with the center swath beginning in the center of on 100 ft side of each plot)
3. "Hopper Blend" - 0.75 lb each of Esteem and Probait® (0.73% hydramethylnon) Ant Bait (Wellmark International) applied at 1.25 to 1.5 lb blended product per acre
4. "Skip swath" of Esteem (0.5% pyriproxyfen), applied at a rate of 1.25-1.5 lb/acre, but with the center swath left untreated and skipping another 20 ft before

applying additional swaths along the outside of each plot (this 2 swaths per plot and an additional 2 swaths along the length of each plot.

5. methoprene - Extinguish® Fire Ant Bait (Wellmark International)

Near Dobbin

1. Untreated control
2. pyriproxyfen (Esteem® Fire Ant Bait) - 1.5 lb/acre
3. "Hopper blend" of 0.75 lb pyriproxyfen (Esteem®) plus 1.0 lb hydramethylnon (Amdro®Pro)
4. "Skip swath" of pyriproxyfen (Esteem®) - 1.0 lb bait/acre applied to every other 25 ft swath
5. hydramethylnon (Amdro®Pro) - 1.5 lb/acre
6. methoprene (Extinguish®) - 2 lbs/acre

Plots were re-evaluated periodically following treatment (at Lake Granger - Nov. 6, 2003, Jan. 14 and April 8, 2004; near Dobbin - July 9, Sept. 14-16, and Dec. 17, 2004, Mar. 8, 2005) as described for the pre-treatment assessment of mound numbers. Data were analyzed for each pre- and post-treatment date using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test at $P \leq 0.05$.

Results. At Lake Granger, 7 months following fall application, mean mound numbers in Esteem® (pyriproxyfen) were finally significantly lower than those in the untreated plots. By June 17 (9 months following application) all but one treatment had significantly less ant mounds than those receiving no treatment; plots treated with the hopper blend did not separate statistically but had 49% fewer mean ant mounds than untreated plots. By September 22 (12 months following application) no significant differences occurred between mean fire ant mound numbers in the 0.51-acre sub-plots.

Near Dobbin, all insecticidal bait treatments significantly reduced ant mound numbers relative to those found in untreated control plots 3 months following June application. As expected, IGR products (pyriproxyfen and methoprene) reduced mound numbers faster than when applied in the fall in the previous 2003 trial. A wet, mild winter resulted in an overall increase in ant mound numbers by the following spring (March 2005), although treatments continued to provide suppression relative to untreated plots. In this trial, re-invasion occurred more dramatically in "hopper blend" treated plots possibly because control after 1 month (July 2004) was very dramatic, leaving very few IGR-treated colonies in plots to prevent re-invasion by newly-mated queens and migrating colonies.

Valent U.S.A. submitted for a pasture registration for Esteem® to the Environmental Protection Agency in June 2004 and anticipates registration by mid-year 2007. More data will be needed before the "skip-swath" application is considered for registration.

Cool-season Applications of Baits and/or Chemical Drenches to Individual Mounds

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Introduction

Imported fire ants (IFA) (*Solenopsis spp.*) pose a threat to airports by infesting electrical installations, chewing on wiring and possibly causing short circuits. Fast acting control methods, such as individual mound treatments, are desirable in these situations. The two-step method, a bait application followed by individual mound treatments with a contact insecticide, is recommended for ornamental turf and non-agricultural lands (Drees et. al. 2002). Spot treatment or individual mound treatment, including drench treatments, have been extensively studied and recommended under specific circumstances for IFA control (Collins and Callcott 1995). In a drench simulation study *S. invicta* was found highly susceptible to different insecticides used for mound drench treatments (Drees 2002). This study was conducted to determine the effect of bait applications on individual mounds during cooler season and to compare individual bait and drench treatments to the combination of two.

Materials and Methods

The study was conducted at the Fayetteville-Lincoln County Regional airport in south-central Tennessee. Mounds used in the study were located at one of the three locations: in open ground, under the airport lights or adjacent to the pavement of the runway or taxiways. Treatments were randomly assigned to mounds and there was at least 9.1 m (30 ft) between mounds. Treatments consisted of Advion™ (Indoxacarb) bait at 4 tablespoons (22 g)/mound, AmdroPro® bait at 4 tablespoons (22 g)/mound, Talstar® GC Nursery Flowable (Bifenthrin) at 0.2 ml/treatment area, Topchoice™ (Fipronil) at 6.4 g/treatment area and GardStar® 40% EC (Permethrin) at 5 ml/treatment area. Each of these was tested individually and in a combination treatment: application of a bait followed by the drenched application of a chemical (Talstar, Topchoice or GardStar) 6 days later. All combinations of bait + drench were used making for a total of 11 treatments. A no-treatment control and a water-only control were included. Each treatment (including controls) was replicated seven times with each replication consisting of a single mound.

Treatment area for drenches was a 0.9 m (3 ft)-diameter circle (0.66 m^2 or 7.07 ft^2) including the mound at the center. Fluid treatments were applied to each circle in 7.6 L of water, with 3.8 L of the mixture applied directly on the mound. Granular Topchoice™ was evenly distributed in the treatment area and irrigated. Baits were evenly distributed in a 2.1 m (7 ft)-diameter circle (3.57 m^2 or 38.48 ft^2) around the mound. The treatment area of each mound was checked for ant activity at 1, 4, 6, 7, 10, 14, 20, 33, 78 and 99 days after treatment (DAT) for bait-only and bait + drench combination treatments. Mounds receiving drench-only treatments were checked at 1, 4, 8, 14, 27, 72 and 93 DAT. Mounds were rated as active or inactive based upon presence or absence of worker activity after disturbing the mound with a metal wire at 4 random places. Average number of days to mound inactivity was calculated for each treatment. Chi-square test was applied to determine the association between treatment and mound activity at 1, 4 and 14 DAT (the only DATs that all treatments had in common) (SPSS 2003). Samples of IFA were taken from randomly selected mounds and identified to species by Dr. Robert Vander Meer, USDA-ARS-CMAVE, using cuticular hydrocarbon analysis. Daily rainfall, soil and air temperatures of the test site were recorded by a WatchDog® data logger (Spectrum Technologies, Inc.) protected in a radiation shield.

Results and Discussion

Of the bait-only treatments, Advion provided better control: 57, 86 and 100% of mounds were inactive at 7, 14 and 33 DAT, respectively. All Advion-treated mounds were still inactive at 99 DAT. On those same days, 43, 57 and 86% of mounds treated with AmdroPro were inactive. Inactivity never exceeded 86% for AmdroPro. Barr (2003) achieved 90% inactivity of *S. invicta* mounds within 9 DAT and 10 weeks after treatment when Advion was broadcast during summer and fall, respectively. Among drench-only treatments, GardStar provided 100% inactivity throughout the observational period. Talstar provided 71, 86 and 100% inactivity at 1, 14 and 27 DAT. Ant activity in Topchoice-treated mounds varied little from that of the water-only control mounds. Advion achieved 100% mound inactivity by 7 DAT when used in combination with Talstar or GardStar. The Advion-Topchoice combination produced 43, 57 and 86% inactivity at 7, 33 and 99 DAT, although a rise in control inactivity occurred at 99 DAT. The AmdroPro-Talstar combination achieved 100% inactivity by 7 DAT. The AmdroPro-GardStar combination produced 71, 86 and 100% inactivity at 7, 10 and 78 DAT, respectively. The AmdroPro-Topchoice combination produced 57% inactivity throughout the observation period. For treatments providing 100% inactivity, average days to inactivity were 7, 7, 7, 7, 3.3 and 12.7 for GardStar, Advion-GardStar, Advion-Talstar, AmdroPro-Talstar, Talstar and Advion, respectively.

The association between mound status and treatment was examined by Chi-square analysis. For bait-only treatments, days 1 and 4 were not relevant because baits do not act quickly. For drench-only treatments there was a significant association at days 1 and 4 because of the performance of GardStar and Talstar. At day 14 there was a significant association for all 13 treatments. No effect of mound location (at runway lights, near pavement or in open ground) on treatment efficacy was detected. Cuticular hydrocarbon analysis indicated the ratio of *Solenopsis richteri* to the hybrid of *S. richteri* and *S. invicta* at the study site was 40 to 60. Efficacy may have been affected by IFA species, but location of specific mounds from which ant samples were taken was not noted. Therefore, effects of species, if any, could not be examined.

Acknowledgments

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Characterization of Delayed Toxicity in Fast-Acting Fire Ant Baits

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Speed of efficacy and the response profiles of fire ant baits that claim to control red imported fire ant, *Solenopsis invicta*, colonies faster than the typical 2-4 week time period were examined. Large laboratory colonies of *S. invicta*, containing approximately 38,000 workers, 42 ml of brood, and 1 queen, were given access to fast-acting fire ant baits containing either 0.045% indoxacarb (Advion) or 0.015% spinosad (Ortho Fire Ant Killer), a standard bait with 0.73% hydramethylnon (Siege-Pro), and a control of 30% once-refined soybean oil on pregeled defatted corn grit. Colonies were either not starved or starved for 5 days, and there were five replicate colonies per starvation regime and treatment.

In general, effects of baits were more pronounced in the starved colonies. Starved colonies exposed to the indoxacarb bait had 100% worker mortality and all queens were dead within 3 days, while non-starved colonies reached the same level of control in 6 days. The standard bait containing hydramethylnon had 100% of the queens die within 9 days and 83% worker death when colonies were starved. In contrast, colonies that were not starved had 60% queen mortality and a maximum of 36% worker reduction. Colonies exposed to spinosad over both starvation regimes had a maximum worker reduction of 35% and one queen dead after 18 days, while control colonies grew larger without any queens dying.

The mortality response profile of *S. invicta* workers from a 24 hour exposure to the baits showed that only hydramethylnon nearly met the traditional delayed toxicity criteria, as defined by Stinger et al. (1964), of <15 % mortality at 24 hours after initial access to toxicant and ≥90% mortality by 14 days. Spinosad killed 96% of the workers and indoxacarb killed 57% within 24 hours. However, the response profile for indoxacarb revealed virtually no symptoms of toxicity for at least 8 hours before an abrupt, rapid increase in mortality which reached 100% in 48 hours. This contrasted with spinosad which caused very rapid mortality that started within 4 hours and by 48 hours had 99% mortality. Given the consistent and rapid laboratory colony death with the indoxacarb bait, Stringer's delayed toxicity criteria perhaps should be modified to include toxicants that provide delays of at least 8 hours before the onset of symptoms of toxicity. A shorter delayed toxicity criterion could facilitate the development of fast-acting fire ant baits.

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Effect of red imported fire ant baits on some of the non-target ants.

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Background:

Baits remain one of the major approaches to the management of the Red Imported Fire Ant (RIFA), *Solenopsis invica* (Williams et al., 2001). Among some of the more widely used baits contain the products Hydramethylnon and sulfuramid: Example. Amdro®, Combat®, and Maxforce®. Another group of management compounds are the Insect growth regulators that were shown to slowly reduce the reproduction of the colony (Vinson and Robeau 1974) and analogues were later developed and have been used in management (Drees et al., 2002). Some of the current insect growth regulators are Logic® and Distance®.

The purpose of this study was to determine if these oil based toxicant baits used in RIFA management programs could have an impact on some of the more important competing native ants (see: Rao and Vinson, 2002; Helms and Vinson, 2001; Nichols and Sites, 1991; Nickerson et al., 1975). The objectives were to determine if the current oil based baits were attractive to and acceptable to non-target native ants? The next question was: Do the RIFA baits affect non-target native ants? To answer this we studied two species of non-target ants: these were the little black ant, *Monomorium minimum* and the Thief ant, *Solenopsis* (*Diplorhoptrum*) *molesta.* These were chosen because we had evidence that they can not only be competitors of the RIFA at food resources (Vinson, Personal observations), but they are capable of invading small colonies of the RIFA and kill the queen (Rao and Vinson, 2004).

MATERIALS AND METHODS

We examined the effects of several commonly used baits on 4 species of ants. These included the intended target ants the imported fire ant, *Solenopsis invicta* Buren, and the household pest, Pharaoh ant, *Monomorium pharaonis*. For the tests presented here, we tested 2 baits used in RIFA management and we had two controls. The controls consisted of insect food

(Crickets and Meal worms) and a diet prepared according to Kuriachan and Vinson (2000). We used two baits Amdro® (containing Hydramethylnon) and Logic® (containing an insect growth regulator). We used two controls, one was a bait formulation similar to the two baits used above, but with out any toxicant, the other control consisted of the same insect food described above.

Prior to the treatments small experimental colonies were formed from field-collected colonies that were reared in the laboratory for 1 to 2 months. These colonies consisted of 1000 randomly selected workers, 2 queens, and brood equal to 5 times wt of a single queen. These colonies were placed in a shoebox with a castone nest and water tubes and they were held for one week. Prior to allowing access to the baits or food, the colonies were starved for 48 hours. To provide access to the bait, 250 mg of toxic bait (or control food) was weighed and placed into a small dish that was placed in another shoebox at one end. The test began when the ant colonies were provided a paper bridge from their nest container to the box with the bait, but one end of the bridge was placed in the nest box at the end of the box furthest from the nest and the other end was placed in the bait box furthest from the bait. We also marked a 2 X 2 inch square area around the bait, a "feeding zone".

To evaluate bait acceptance, we counted the number of ants within the "feeding zone" at 15, 30, 45 and 60 min. after bait placement. We also determined the amount of bait or food consumed by determining the difference in the weight of the resource before introduction and after its removal at 24 hrs. Colonies were returned to a daily feeding regime and the number of ants that died were counted daily for two weeks and then weekly for two months. There were 4 replications and the recruitment to the bait was analyzed using MANOVA-RM. The worker mortality was calculated (log-transformed on sampling-times) and expressed as cumulative worker mortality that was analyzed using one-way ANOVA.

RESULTS AND DISCUSSION:

Analyzing for between-subject effects (MANOVA-RM), we found no significant treatment effect on number of ants recruited to different baits. Looking at within-subject (repeated) effects, we found a significant effect of "time" on number of ants recruited (Fig. 1), but no significant effect of the "bait x time" interaction. Looking at recruitment (Fig. 1) the number of RIFA recruited was much lower that the other species. This was due, in part, to the fact that the RIFA's picked up the bait and took it back to the nest. As a result they did not stay long and removed the bait faster (data not shown).

In contrast, the other species were smaller than the RIFA and appeared unable to pick up the bait and return to their nest until it was carved up and most of the lipid was removed. As a result they stayed at the bait longer removing the liquids and more workers were recruited.

There was also no significant bait effect on the mean amount of toxic bait (± SEM) removed in 24 hrs (ANOVA results), as most of the bait was all removed by all the species before the 24 hr period ended. Regarding the effects of the baits on the different ants (Table 1), Amdro resulted in significant mortality for all four species over the two controls and over Logic. However, Logic did result in a significantly higher mortality for all four species than the two controls. Although not shown here, the Amdro did result in near complete mortality of all the species except *M. minimum* in four weeks, while it took eight weeks to kill the *M. minimum* colony. The Logic only began to show significant mortality in 4 to 8 weeks over controls for all four species. In summary it appears that both native ants, *S. molesta* and *M. minimum,* were recruited to the baits and responded in a similar manner as both *S. invicta* and *M. pharaonis.* Further, the more rapid recruitment of both *S. molesta* and *M. minimum* suggests that even in competition with *S. invicta* they may obtain enough toxicant to have a detrimental effect on the colony health.

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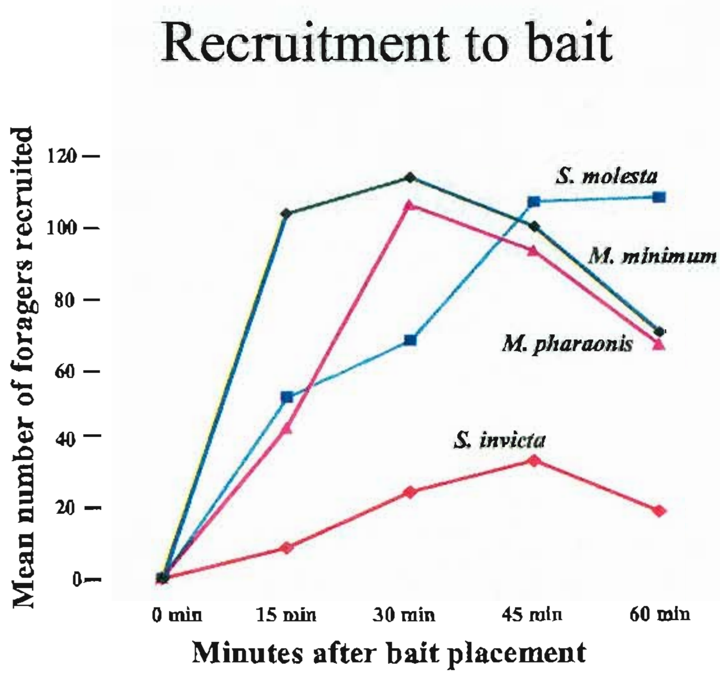

Figure 1, Recruitment of four species of ants to all the baits (there was no significant difference between the baits for a given speciesat a given time).

Table 1. Mortality of workers exposed to two toxic baits and two controls. Columns with the same letter are not significantly different.

Bait type	Mean cumulative worker mortality for species indicated			
	S. invicta	*S. Molesta*	*M. pharaonis*	*M minimum*
Amdro	692.1±17.1a	672.7±13.0a	659.0±16.6a	516.9±8.5a
Logic	71.0±13.8b	67.9±12.9b	70.9±15.4b	51.3±8.5b
Control bait	35.2±13.8c	17.9±12.9c	24.8±15.4c	19.1±8.5c
Regular diet	36.0±13.8c	17.9±12.9c	23.1±15.4c	17.3±8.5c

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APHIS Regulatory Overview

The Imported Fire Ant (IFA) Quarantine is administered by the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture. The goal of the Federal Quarantine is to prevent the artificial spread of IFA. Although IFA have currently become established in 13 States and in Puerto Rico, many other regions and States in the Continental U.S. and in Hawaii have conditions where new IFA infestations could survive.

The IFA Federal Quarantine was established in 1958. The quarantine began as a cooperative eradication program in 9 States (Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina, and Texas). When converted to today's dollar amounts, the initial funding for the quarantine was about $17 million. As several insecticides were developed and shown to be effective against IFA, funding for the quarantine reached $37 million in 1971. However, environmental and human health concerns eventually led to those insecticides being banned from use in 1977. Congressional support then waned until 2000 when only $113,000 was appropriated for the IFA Quarantine.

Through the efforts of the National Plant Board, the nursery industry, and the public in uninfested states, funding for the IFA Quarantine was restored in 2001 and is currently just over $2 million. These funds are primarily used to regulate articles that pose a risk of spreading IFA to uninfested areas and for surveys to determine the extent of IFA spread. Among the articles specifically regulated in 7 CFR 301.81 (Code of Federal Regulations) are the movement of soil, baled hay and straw, plants and sod with roots and soil attached, soil moving equipment, and any other article that presents a risk of spreading IFA. Information on the IFA Quarantine can be found at: http://www.aphis.usda.gov/ppq/ispm/fireants/index.html.

Another aspect of the IFA Quarantine is the phorid fly release program that involves APHIS, the Agricultural Research Service, and the Florida Department of Plant Industry, IFA-infested states, and universities. This program is responsible for the rearing and releases of *Pseudacteon tricuspis* and *Psuedacteon curvatus* at 39 sites within the IFA Quarantine area. Of the 27 releases done in 2002-2003 there have been 8 sites where overwintering has been observed. Further information on phorid fly releases can be found at: http://www.cphst.org/sections/SIPS/.

Immersion Treatments for Imported Fire Ant Quarantine with Consideration for Japanese Beetle Grubs

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Introduction

Imported fire ants (IFA) are moving into areas of Tennessee where many producers of field grown nursery stock are located. Approximately 80% of this nursery stock ships outside the Federal IFA quarantine zone (Brooker, J.R., et. al. Trade flows and marketing practices within the United States nursery industry: 1998. Southern cooperative bull. Univ. of Tennessee Agricultural Experiment Station. Knoxville. 2000.), consequently prompting renewed interest in development of new treatments for this stock. Currently, quarantine compliant treatments for harvested balled and burlapped (B&B) plants consist of using a 0.125 lb a.i. per 100 gal water chlorpyrifos solution in either an immersion or in twice daily drenches for three consecutive days. Similarly growers in this area are required to apply a chlorpyrifos immersion at 2 lb a.i. per 100 gal water to comply with state quarantines against movement of Japanese beetle (*Popillia japonica* Newman). A cooperative research effort was therefore initiated with the Tennessee State University Nursery Crop Research Station to screen other insecticides for inclusion in IFA quarantine treatments for B&B with special deference to products effective for Japanese beetle grubs.

Methods

Since the first trial conducted in fall of 2002, a total of seven trials have been completed examining the following twenty-eight treatments against IFA. The table below shows the treatments and which trials they were tested in.

Table 1. Treatments tested in B&B immersion trials from spring 2002 through spring 2004.

Active Ingredient	Product Name	Rate lb a.i./100 gal H_2O	I	II	III	IV	V	VI	VII
Acephate	Orthene 75WP	0.750	■				■		
		0.375				■			
Benzoic acid	Mach 2SC	1.50	■						
		0.75	■						
Bifenthrin	Talstar F	0.230	■	■	■				
		0.115	■	■	■	■	■		
	Bifenthrin Pro	0.050						■	
		0.025						■	■
Carbaryl	Sevin SL	8	■		■				
		4	■			■	■		
Chlorpyrifos	Dursban TNP	2	■	■	■	■	■		
	Dursban 4E	0.1250			■			■	■
		0.0625							■
Deltamethrin	DeltaGard 5SC	0.130	■	■	■				
		0.065				■	■		
		0.040						■	
		0.020						■	
Dimethyl phosphonate	Dylox 80	8	■						
		4	■			■	■		
Imidacloprid	Marathon 60WP	0.40		■					
		0.30	■		■				
		0.20				■	■		
		0.15	■						
Imidacloprid & Cyfluthrin	Discus	0.5 & 0.012					■		
Lambda-cyhalothrin	Scimitar CS	0.034		■	■	■	■	■	
		0.017				■		■	
Thiamethoxam	Flagship 25WG	0.130	■	■	■				
		0.065	■	■	■	■	■		

Trials VI and VII were conducted on plants grown in soil from George Co., Mississippi which is sand loam with an average pH of 6.0. All other trials used plants from Warren Co., Tennessee grown in clay loam soils ranging in pH from 3.5 to 6.0. Root ball diameter averaged 12 inches (30.5 cm) for TN plants and 16 inches (40.6 cm) for MS plants. Upon treatment, root balls were submerged in a solution until saturation, indicated by the cessation of bubbling. The plants were then stored outside in conditions similar to those growers would maintain them under prior to shipping. Details of numbers of root ball replicates in each treatment, number of insects tested, and soil sampling frequency and location varied based on the resources available for each trial and are listed in the table below.

Table 2. Bioassay details of each completed immersion trial.

Trial	Date	Location	IFA				JB	
			B&B replicates	# alates per soil sample	soil sample collection (in days post treatment)	soil sample depths	B&B replicates	# grubs per replicate
I	2002 spring	TN	4	20	60, 120, 180	top 10 cm	8	9
II	2002 fall	TN	4	20	15, 30, 60, 90, 120, 150, 180	top 10 cm	10	*
III	2003 spring	TN	4	20	60, 90, 120, 180	top 9 cm and center 5 cm	10	5
IV	2003 fall	TN	4	20	15, 30, 60, 120, 180	top 9 cm and center 5 cm	10	5
V	2004 spring	TN	4	5	15, 30, 60, 120, 180	top 9 cm and center 5 cm	10	5
VI	2003	MS	3	20	30, 60, 90, 120, 150, 180	top 10 cm and center 7 cm	**	**
VII	2004	MS	6	5	15, 30, 60, 120, 180	5 cm - 15 cm	**	**

* Natural infestation of JB grubs was attempted in the fall 2002 trial.
** Mississippi is not infested with JB so no grubs were tested at this location.

Soil core samples were collected from the root balls designated for IFA testing and kept frozen until bioassays could be conducted at the USDA, Soil Inhabiting Pests Section (SIPS) facility in Gulfport, MS. Wild collected female alate fire ants were exposed to sample soil for a period of fourteen days in 6.35cm x 6.35cm square plastic pots (http://www.hummert.com). A layer of Labstone dental plaster (Nowak Dental Supplies, Inc. 8314 Parc Place, Chalmette, LA 70044) in the bottom of the pots both prevented alate escape through drainage holes and permitted moisture to wick into the bioassay pots from the peat moss bed upon which they sat. Petri dish lids prevented alates from crawling out of the bioassay cups during the test. The contents of each pot were inspected for alate mortality multiple days during the 14 day trial.

Beetle larvae were introduced into the root balls designated for Japanese beetle bioassays prior to chemical treatment. These root balls underwent the same treatment and storage as those used for

IFA testing. Upon evaluation these root balls were then split open at either two months (spring trials) or four months (fall trials) to determine the number of surviving grubs.

Results and Discussion

A successful treatment for quarantine purposes is one that can consistently ensure the commodity will be free of the specified pest when shipped. While reinfestation of a commodity with JB is limited to a specified adult flight season (June - October), IFA may move a colony or have a mating flight any time of the year as long as local weather permits. Thus for IFA the treatment must eliminate any existing infestation and for a consistent minimum period of time prevent establishment of new infestations.

Chlorpyrifos was included in these trials as a standard of treatment activity for both IFA and JB. The IFA treatment rate of 0.125 lb a.i. is the only rate of chlorpyrifos that has tested in both TN and MS soils. Soil type appears to cause extreme differences in longevity of chemical effectiveness of this treatment. The single test at this rate in TN soil showed a decline in efficacy starting at two months after treatment (Table 3), while the two trials in MS soil yielded control of IFA through the end of the trials (six months). Furthermore a rate of 0.0625 lb a.i. was tested in the MS sand loam soil and also lasted through the trial's end.

Surprisingly the IFA rate (0.125 lb a.i.) also provided 100% control of the JB grubs. Further testing of JB at this rate is currently underway. If this lower rate proves to produce consistent control of JB in future trials, the potential benefits of a lower certification rate for this pest would be considerable. The current 2.0 lb a.i. treatment rate produces phytotoxic responses in several plant species. A reduction of rate could thus not only reduce cost of treatment and environmental impact, but also improve plant quality and increase the scope of plant species that could be shipped.

The JB rate of 2.0 lb a.i. performed as expected providing control of both pest organisms with control of IFA lasting a minimum of three months. This indicates that not only can root balls treated for JB quarantine be considered to have been treated for IFA, but also at this rate the period of IFA quarantine certification could potentially be extended from the current limit of one month to three months.

Bifenthrin has performed with ideal quarantine consistency. Regardless of soil type, soil sample depth, or rate all tests against IFA with this chemical have delivered 100% control through the duration of the trials. The four trials of this chemical at the 0.115 lb a.i. rate conducted against JB produced control identical to the 2 lb a.i. chlorpyrifos certification treatment. SIPS is currently pursuing inclusion of a bifenthrin immersion treatment at a 0.115 lb a.i. rate in the permitted quarantine treatments for B&B stock. Because six or more months of control is generally longer than a grower would keep harvested stock, further testing will continue to examine lower rates to determine a time scale of residual activity based on rate of active ingredient.

Deltamethrin immersions at both 0.13 and 0.065 lb a.i. and lambda-cyhalothrin at 0.034 lb a.i. have performed well providing months of IFA control. However, each has had an anomalous result in one or more trials where incomplete control occurred in one sample but 100% mortality was achieved in soils from subsequent sample dates. Further testing of these treatments against IFA is required to determine if consistency can be realized. The previously mentioned rates of deltamethrin have been tested against JB in two trials each. The lower rate eliminated all grubs in both trials, but the higher rate failed to meet that goal. Although lambda-cyhalothrin tested well against IFA, it did not control JB grubs and will not be included in new JB trails.

The remaining treatments not discussed in the above paragraphs either had not completed more than one trial at the time of this presentation or have failed to yield consistent 100% control of IFA. Full details for each treatment tested in IFA trials are available on line in the corresponding SIPS annual reports at http://www.cphst.org/sections/SIPS/annual_reports.htm.

Table 3. Treatments across the seven immersion trials from spring 2002 to spring 2004 with the most promise as a control for one or both pest species are displayed in this table. Japanese beetle grub (JB) survival was sampled once in each trial, so the % mortality is displayed. Imported fire ants (IFA) were subjected to soil exposure multiple times in each trial, so the sample date of the last quarantine acceptable reading is listed under each trial. Treatments favorable for JB that never attained 100% mortality in IFA are indicated by "not" under the appropriate trial. Trials with two soil depths sampled for IFA testing list both end sample dates of 100% mortality in order (upper, center).

Treatment	JB % mortality				IFA months of 100% mortality						
	I	III	IV	V	I	II	III†	IV	V	VI††	VII
Acephate 0.75	91.3	100			not				not		
Benzoic acid 1.5	97.8	100			not						
Bifenthrin 0.115	97.8	100	100	100	6	6	2, 6	6, 6	6, 6		
Carbaryl 4.0	91.3	100	100	100	not			not	not		
Chlorpyrifos 2.0	97.8	100	100	100	4	6	2, 3	6, 6	4, 4		
Chlorpyrifos 0.125		100					1,1‡			6, 6	6
Deltamethrin 0.065			100	100				6, 6	3, 5*		
Dimethyl phosphonate 4.0	97.8	100	100	100	not			0.5, 0.5	not, 1		
Lambda-cyhalothrin 0.034		68	89.2	53		6	2, 3	6, 6	6*, 6*	6*, 6*	
Thiamethoxam 0.065	97.8	100	67.6	100	2	3	not, 1‡	0.5, 6	1, 1		

† Trial III had a top 9cm sample drawn only at 60 days post treatment; center 5cm samples were collected at all dates in this trial.

‡ The first soil sample collected in trial III was at 60 days. Those treatments which had more than 90% but less than 100% mortality at this relatively late first sample date are presumed to have had 100% at 30 days after treatment.

†† Trial VI had a center 7cm sample drawn at 90 and 180 days post treatment; top 10cm samples were collected at all dates in this trial.

* Indicates an anomalously low reading from one replicate from a sample date prior to the final 100% mortality reading in that trial.

Establishing guidelines to improve identification of the fire ants, *Solenopsis xyloni* and *S. invicta* by: A.L. Jacobson, D.C. Thompson, and L. Murray, New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Box 30003, MSC 3BE, Skeen Hall W220, Las Cruces, NM 88003

This is the abstract of the talk we presented. The complete paper has been submitted for publication in the Journal of Economic Entomology.

Doña Ana County, New Mexico was quarantined in 1999 for red imported fire ant, *Solenopsis invicta*. This very aggressive ant species displaces native ant populations, disrupts natural ecosystems, interferes with agricultural operations, and is a health hazard to livestock, wildlife and people. Despite regular surveys, *S. invicta* hasn't become dominant in the county during the past seven years. In fact, only two populations of *S. invicta* have been confirmed since the initial quarantine. The predominant fire ant is the southern fire ant, *S. xyloni*. One speculation is that the native *S. xyloni* was initially misidentified because variation of distinguishing characteristics is common within this species. Variability of these characteristics was quantified by conducting a morphological comparison of 10 individuals from 10 colonies of both species using scanning electron microscopy (SEM). The clypeal tooth, striations of the mesopleuron, length of antennal scape, the petiolar process, number and size of mandibular teeth, and color were examined. All of these characteristics are different between the two species; however, significant morphological variability occurs in both *S. xyloni* and *S. invicta* populations creating an area of overlap where either of the two species could exhibit the

characteristic. Better differentiation of these two species is achieved using a combination of characteristics. Due to the overlap of important characteristics and the difficulty visualizing these characteristics, care should be taken when identifying these two species in areas where they coexist. For situations requiring an absolute identification such as prior to quarantining a county or a portion of a county, a molecular technique using mitochondrial DNA and PCR techniques was developed.

Carol Russell

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RIFA Pest Detection Efforts in Hawaii and the Pacific

Addressing efforts in Hawaii and throughout the Pacific region, requires better prevention measures for possible incursions of red imported fire ants. Hawaii and the Pacific Island Countries and Territories (PICT) are known for their island isolation and fragile ecosystem. An incursion of red imported fire ants could spread rapidly and go undetected.

Through regulation set by Hawaii's state quarantines, detection in Hawaii is monitored by the Hawaii Department of Agriculture at entry points in to the state from the U.S. mainland. Hawaii Department of Agriculture has been meeting with other organizations regarding working collaboratively on efforts in detecting RIFA. Cooperative Agriculture Pest Survey through the USDA/ APHIS/ PPQ provides funding to the Hawaii Department of Agriculture for pest survey programs for RIFA.

Known as an invasive species, RIFA in Hawaii will be a targeted pest for a Report A Pest Media Campaign – kickoff in June 2005, beginning with public service announcements.

In the Pacific, a workshop was held in New Zealand in 2003 to initiate a regional approach to prevention. It resulted in the compilation of a draft Pacific Ant Prevention Plan (PAPP) that encompassed RIFA and other exotic invasive ants that have demonstrated negative impacts. Given the eradication difficulties of many invasive alien ant species (esp RIFA) obviously prevention of entry is of utmost importance and the highest priority. The PAPP proposal outlines the necessary framework for both the prevention of entry and the prevention of establishment. As of March 2004, the Pacific Plant Protection Organization and Regional Technical Meeting for Plant Protection was held in Suva, Fiji. The plan was adopted by the PICT.

As of 2005, there has been much progress with this plan including funding and identifying a coordinator position. A list server has also been created that provides a good forum that extends out Pacific wide for ants.

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Interaction between *Monomorium minimum* and healthy or *Thelohania solenopsae* infected *Solenopsis invicta*: a laboratory study.

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Background:

The Red Imported Fire Ant (RIFA) *Solenopsis invicta* has invaded much of the Southeast US and is invading many areas around the Pacific Ocean (Callcott and Collins, 1996; Dowell and Krass, 1992, Moloney and Vanderwoude, 2004). This invasion has been at the expense of some native ants (Gotelli and Arnett, 2000; Hung et al., 1978; Jusino-Atresino and Phillips, 1994; Porter and Savignano, 1990), although some species persist (Helms and Vinson, 2001) or may return (Morrison, 2002). But, there is little evidence that these native ant species can eliminate RIFA colonies once they consist of a few defending workers. We do know that a number of native ants can be predators of newly mated RIFA queens as they land or soon after they land (Lammers, 1987; Nichols and Sites, 1991; Nickerson et al., 1975; Whitcomb et al., 1973). It has also been reported that a number of native ants can persist in RIFA infested areas (Helms and Vinson, 2001; Stein and Thorvilson, 1989; Wojcik, 1994). In addition it has also been shown that in a laboratory setting that some native ants can invade and will kill small RIFA colonies (Rao and Vinson, 2004). Further, small RIFA colonies were unable to establish in an area containing a large population of the thief ant, *Solenopsis (Diplorhoptrum) molesta* (Vinson and Rao, 2004). Others have also suggested that thief ants may prevent the invasion by the RIFA (MacKay and Vinson, 1989) into areas that they dominate.

Presently there is a lot of interest in developing a biological control approach to RIFA management. A large number of diseases and parasitoids or parasites of the RIFA are known (Jouvenaz 1983, 1986, 1990) and a number have been considered for management. These include the strepsiptera (Cook et al., 1995), the fungus *Beauveria* (Bextine and Thorvilson, 2002; Siebeneicher et al., 1992), nematodes (Drees at al., 1992), and more recently Phorid flies (Gilbert and Patrock, 2002. Mottern et al., 2004). The microsporidian *Thelohania solenopsae* Knell, Alan, and Hazard is of particular interest because it has been reported that this cellular parasite can kill colonies of the RIFA if given a number of months (Knell et al., 1977; Oi and Williams 2002; Williams et al., 1999). However, we wanted to determine if *Thelohania* infected colonies were more susceptible to invasion by some native ants and chose to work with the native ant, *Monomorium minimum.*

Objectives:

We developed 3 objectives. 1). Compare the ability of the RIFA and *Monomorium minimum* to invade each other. 2). Confirm that *Monomorium minimum* is deterred from invading RIFA colonies of increasing worker number. 3). Determine if RIFA colonies infected with *Thelohania solenopsae* are more or less susceptible to invasion by *Monomorium minimum* than healthy RIFA colonies.

Materials and Methods:

RIFA colonies utilized in these experiments were collected from two sites in Brazos County, TX; two sites in Burleson County, TX; and one site in Guadalupe County, TX. RIFA mounds were collected in buckets, removed from the soil by flotation and maintained in the laboratory as described by Banks et al. (1981). *Monomorium minimum* colonies were collected from two sites in Brazos County, TX and 2 sites in Tom Green County, TX. The *M. minimum* colonies were collected in from rotten logs that were brought into the laboratory, broken apart, placed in a large tray and allowed to dry out. Cotton plugged water tubes were placed in the tray into which the *M. minimum* colony moved.

Once ants were removed from the soil or wood they were placed in plastic shoe or sweater boxes with food (insects), and water. For the RIFA colonies, their infection status was determined by grinding about a hundred ants in saline and staining using Trichrome stain (Weber et a., 1992; Milks et al., 2004).

Prior to initiating experiments 2 plastic shoeboxes were taken and the long side placed 9 cm next to each other. Next three 10 cm glass tubes were inserted between the 2 shoeboxes at 5, 15, 25 cm from edge and 1 cm from bottom to form a tunnel between the 2 boxes. These were plugged.
Prior to initiating the experiments using *M. minimum* colonies; three dealate queens, 1000 workers and brood equivalent about 300 workers were placed in one of the paired plastic sweater boxes coated with Fluon®. Each box was provided with a 20 ml test tube filled half way with water and corked

with a cotton ball and served as nests. In the case of the non-infected RIFA colonies, 5 different sizes were formed from some of the field-collected nests to be paired with the one size of *M. minimum* colony. These RIFA colonies consisted of 2 dealate queens and 100, 300, 600, 800, or 1000 workers randomly chosen without regard to worker size, and brood was added equivalent to about a third of the number of workers that was added. The same procedure was repeated for the *Thelohania* infected RIFA colonies. These colonies were allowed to acclimate for 24 hr. before the invasion was initiated. To start the invasion the cotton plugs were removed and wooden toothpicks were placed in entrances to the tubes. All parings were replicated 5 times (involving 50 *M. minimum* colonies, 25 healthy RIFA colonies, and 25 *T. solenopsae* infected RIFA colonies).

Observations for the experiments reported here consisted the entrance of one species into the other species box, on the other species nest and entering the other species nest. Observations were made every hour for first 12 hours and thereafter once daily for 31 days.

Results:

In general the *M. minimum* was first to invade in almost all cases and engaged in combat with RIFA workers encountered by raising her abdomen and spraying venom towards the RIFA. The foraging *M. minimum* were also the first to enter the nest of their competitor, the RIFA. The RIFA generally retreated to their nest or only defended against the invaders within their nest. The venom appeared to reduce the ability of the RIFA to defend itself. Fig 1 shows the results of the invasion of *M. minimum* into the different sizes of healthy RIFA nests. There was no significant difference (ANOVA, when $\alpha = .05$) for *M. minimum* to invade the RIFA box containing different numbers of healthy fire ants. In contrast, there was a significant difference (ANOVA, when $\alpha = .05$) between the ability for *M. minimum* to forage on or to enter the different sizes of healthy RIFA nests.

Fig 2 shows the results of the invasion of *M. minimum* into the different sizes of *Thelohania* infected RIFA nests. There was no significant difference (ANOVA, when $\alpha = .05$) for *M. minimum* to invade the RIFA box or between the ability for *M. minimum* to forage on or to enter the different sizes of *Thelohania* infected RIFA nests. As shown in Figure 3, there was no difference in the ability of *M. minimum* to invade the nest containing box or foraging on the nest or entering the nests of healthy or infected colonies of 100 workers. However there was a significant difference in the ability of *M.*

minimum to invade the nest containing box or foraging on the nest or entering the nests between healthy and infected colonies of 1000 workers.

Discussion:

The results of this study showed that the RIFA colony size affects the ability of *M. minimum* to invade healthy RIFA colonies and confirms the earlier results of Rao and Vinson (2004) that small RIFA colonies are susceptible to invasion by some of the larger colonies of native ants while larger RIFA colonies are more resistant. The results also demonstrate that the colony size of infected RIFA does not have a significant effect on the ability of *M. minimum* to invade and suggests that the stress caused by *T. solenopsae* infection can negatively affect the ability of RIFA to defend their nest. This may apply to several other native ant species that were found to be capable of invading small RIFA colonies (Rao and Vinson (2004). This study also provides some evidence that combinations of bio-logical control strategies can lead to better management of the RIFA problem and that native ants may play an important role (Drees et al., 1996).

Acknowledgments:

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Figures (1-3):

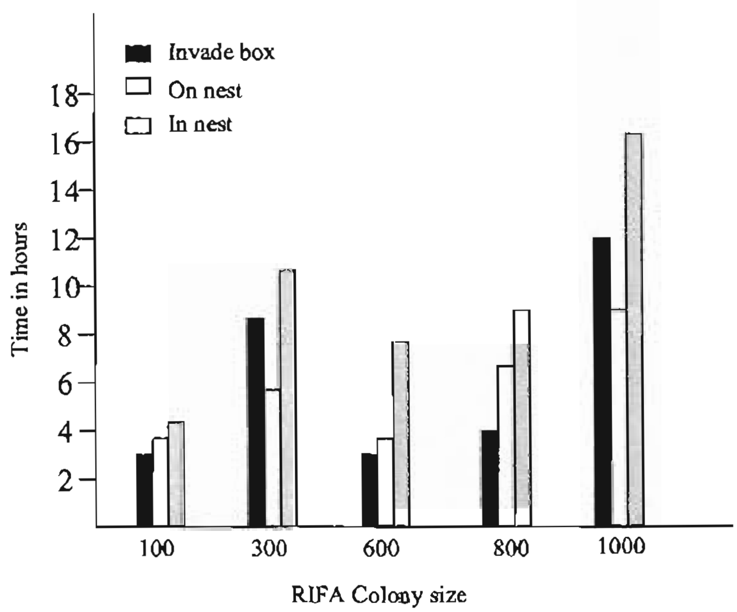

Figure 1. Invasion of nests of healthy RIFA by *Monomorium minimum*

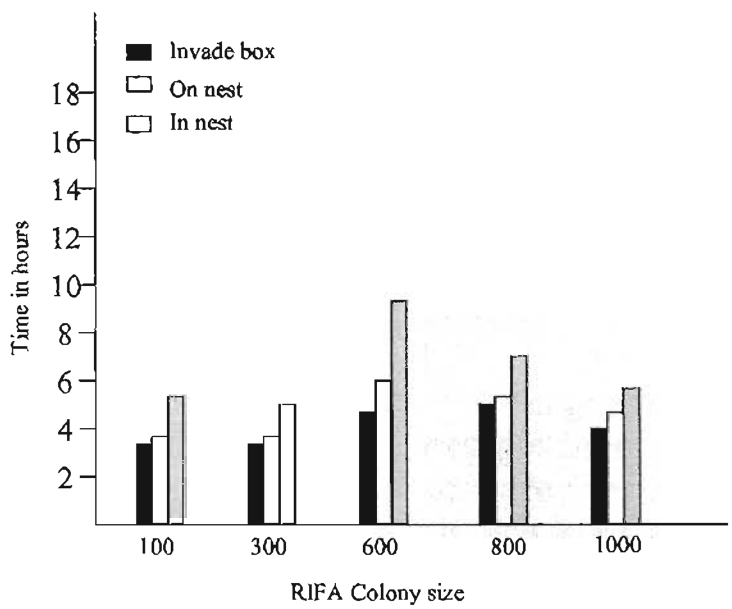

Figure 2. Invasion of nests of *Thelohania* infected RIFA by *Monomorium minimum*.

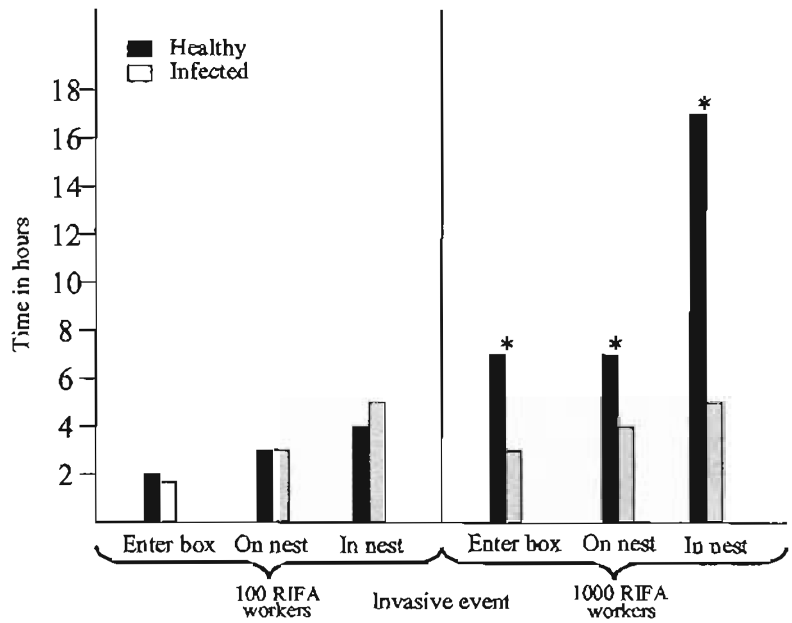

Figure 3. Comparison of the invasion of two sizes of infected and healthy RIFA nests by *Monomorium minimum*. * = Significant difference.

Co-Occurrence of Solenopsis invicta with native ant species in South Carolina

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ABSTRACT

Pitfall sampling was conducted at 243 sites throughout South Carolina in 1999 and 2000. Sampling was stratified by physiographic region: mountain, piedmont, sandhill and coastal plains (Barry 1980). Sampling was further stratified by landcover type as classified by the SC Gap Analysis Program. A total of 41,414 individual ants were captured, counted, and identified. Distributions for a total of 65 species were modeled. A predicted species richness map was generated by adding the distribution maps using GIS software. Species richness ranged from zero to 41 species. These data were used to perform a telescopic view of the species that co-occur with RIFA. Correlations were performed of presence absence of species collected against the presence of fire ants. The following correlations were determined to be significant at the scale of the collection.

Species	r2	$P < 0.05$
Apheanogaster rudis	-0.310	0.000000835
Brachymyrmex sp	0.268	0.0000250
Camponotus floridanus	0.186	0.00371
Cyphomyrmex rimosus	0.465	2.425x10-14
Hypoponera opaciceps	0.155	0.0163
Paratrechina arenivaga	0.187	0.00349
Paratrechina concinna	0.233	0.000257
Paratrechina faisonensis	0.405	6.466x10-11
Paratrechina vivdula	0.217	0.000707
Prenolepis imparis	-0.132	0.0400
Solenopsis pergandei	0.252	0.0000741
Tapinoma sessile	0.179	0.00535

Diversity and Interactions of Ant Assemblages with the red imported fire ant in Pecans

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Local diversity, population dynamics and interactions of ant assemblages were studied in a pecans in Mumford, Texas. The assemblages included the red imported fire ant (RIFA), *Solenopsis invicta* Buren. The study addressed the question of what is the response of the ant assemblage after reducing RIFA following insecticide applications (bait and contact insecticide) and the impact of these on individual species. To approach this question, three treatments were established in a 16 hectare area in the orchard. The treatments were randomly assigned in 1.33 hectare blocks with four replications and periodically monitored. Treatments were: 1) insect growth regulator (IGR) bait treatment (Extinguish^{TM}, 0.5% s-methoprene) applied twice in 2000 and once in 2001; 2) chlorpyrifos (Lorsban^{TM}, 44.9% chlorpyrifos) applied on tree trunks four times in 2000 and once in 2001; and 3) untreated Control. Blocks were sampled using pitfall traps, baited vials, direct sampling, and colony counts. Data were analyzed by using ANOVA-GLM with the LSD multiple comparison test to compare the effect of treatment on the ant assemblage (using the Shannon index) and the effect on individual species. The ant assemblage consisted of 16 ant species. *S. invicta* was the most abundant followed by *Paratrechina sp.* and *Monomorium minimum*. The IGR treatment consistently reduced RIFA (77%). Native ants were found to coexist with RIFA in the Control and chlorpyrifos plots at lower densities and maintained higher densities in IGR plots. Chlorpyrifos trunk treatment did not have a significant impact on RIFA or native ant densities. The native ant, *Dorymyrmex flavus*, was greater in IGR plots following RIFA reduction and higher densities were found to persist for more than two years after the last IGR treatment.

RED IMPORTED FIRE ANT IMPACT ON NATIVE ANTS AND LITTER REMOVAL IN THE POST OAK SAVANNAH OF CENTRAL TEXAS: HIGHLIGHTS OF A MASTER'S THESIS

Theresa L. Bedford, William E. Grant, and S. Bradleigh Vinson

The red imported fire ant (RIFA; *Solenopsis invicta*) is a successful invader which alters species composition and impacts community function. In our study, we examined the impact of RIFA on litter removal in a grassland ecosystem by assessing and comparing the amount of ground litter removed by RIFA and native ants in 3 areas of differing RIFA densities. The hypothesis tested was that litter baits will be removed in equal amounts by both RIFA and native ants in areas with naturally occurring RIFA densities, areas from which RIFA densities have been experimentally reduced, and areas with artificially high RIFA densities.

The study area was located in the post oak savannah 11 km south of College Station, Texas. Three adjacent areas in a 0.45 ha meadow were flagged in a 2.5- by 2.5-m grid pattern, to facilitate ant population surveys and ant density treatments. Two ground litter removal stations were located in each area. Ten RIFA colonies were transplanted into the high RIFA density area, using specially designed buckets.

Ant population surveys were conducted weekly in late spring, summer, and early fall, and monthly in winter, from July 1998 to October 1999. Vials containing 2 pellets of Happy Cat® were placed at the bases of the grid flags. After 15 minutes, the vials were collected, transported to the lab, and then frozen for 24 hours. The ants in each vial were counted and identified, and catch per unit effort (CPUE) was calculated.

During the ant surveys, RIFA was treated in the low RIFA density area by replacing a retrieved vial containing RIFA with a vial half filled with Amdro®. The Amdro® vials were collected after 30 minutes and disposed of. Native ants were treated, in a similar fashion, in the high RIFA density area, until October 1998, when the transplanted RIFA colonies were added.

Litter removal stations consisted of 3 6-oz "tin" cans with screen mesh coverings. A square of wire mesh was placed beneath each can to keep vegetation away from the can lip. A large spike was hammered through each can bottom to hold everything in place. Silicon stripping sealed any gaps.

Fourteen ground litter removal trials were conducted from 21 September to 21 October 1999. Crickets, mealworms, or mixed seeds were placed in weighboats, weighed to nearest 0.0001 g, transported to the study area, and placed inside the litter removal station cans. Twelve hours later, the bait weighboats were removed, and notes were taken on ant species seen in the cans. In the lab, the remaining baits were frozen, dried at 60°C for 24 hours, cooled and weighed to nearest 0.0001 g. These remaining bait masses were converted to wet weights, and means were calculated

During the population surveys, RIFA, *Monomorium minimum*, *Paratrechina* sp. (*P. vividula* or *P. terricola*), S. (*Diplorhoptrum*) *krockowi*, *Pheidole metallescens*, *Forelius pruinosus*, and *Camponotus americanus* were collected. More than 99% of the ants were either RIFA (65.1%), *M. minimum* (30.1%), or *Paratrechina* sp (4.2%). The ant population surveys show seasonal variation in the ant populations CPUEs.

Of the 126 cans of bait used during the study, RIFA was noted in 48 cans, *M. minimum* in 7 cans, *Paratrechina* sp. in 4 cans, and *F. pruinosus* in 1 can. Two cans contained multiple species, and were not used in the mean calculations.

The mean mass of litter removed per can by RIFA was higher in the high RIFA density area, at 0.75 g (n = 19), than in the naturally occurring density area, at 0.42 g (n = 29). In the low RIFA density area, *M. minimum* removed 0.16 g (n = 7) of litter per can, *Paratrechina* sp. removed 0.12 g (n = 2) per can, and *F. pruinosus* removed 0.22 g (n = 1). *Paratrechina* sp., removing 0.15 g of bait per can, (n = 2) was the only native ant seen in the high RIFA density area cans. No native ants were in naturally occurring RIFA density litter removal cans, while no fire ants were observed during collection of bait from the low RIFA density area litter removal stations.

This study indicates that since litter baits were not removed in equal amounts by both RIFA and native ants in the 3 RIFA density areas, RIFA does have an effect on habitat use by native ants.

We would like to thank The State of Texas Red Imported Fire Ant Research and Management Plan for funding this study.

Imported fire ants (*Solenopsis* spp.) in forested landscapes

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Introduction

Forested landscapes are dominated by forest but also typically include some open areas such as roadsides, woods roads, trails, wildlife food plots and open areas associated with thinnings and/or clearcuts. Within forested landscapes, imported fire ants (IFA) are most common in open areas, but can also be found occasionally within the forest itself (Morril et al. 1978, Tschinkel and Hess 1999). We are beginning to evaluate the mechanisms influencing fire ant distribution patterns in both open and forest habitats of forested landscapes. Here we present the details of our project that is directed at occurrences of IFA inside of a forest.

The probability of an IFA colony occurring at any given point within a forest is related to two groups of factors, site factors and landscape factors. Site factors are local conditions that effect the colony's ability to establish and survive. Some examples are the presence or absence of other arthropods that may interact with the fire ants, or physical conditions such as temperature, sunlight and soil characteristics. Landscape factors are large-scale factors that effect the probability of fire ants reaching any location. Some examples of landscape factors are landscape composition, landscape configuration and landscape connectivity. Landscape composition is the relative proportions of different habitat types within the landscape. If open habitats are preferred by IFA, then the amount of open area in the landscape surrounding a point in the forest could influence the probability of IFA reaching that point. Landscape configuration is the arrangement of land cover types. A landscape that is 70% forest could include one large patch of forest or several smaller patches. Smaller forest patches have a greater perimeter to area ratio, will have more edge habitat, and may be easier for IFA to penetrate than larger patches. Landscape connectivity is the degree to which the landscape is connected from the perspective of the fire ant. Landscape connectivity is related to the composition and configuration of the landscape and can also be influenced by the presence of roads or other corridors.

Part I: Factors associated with the occurrence of imported fire ants in forests

Part I is a survey of random points in forests and of locations with fire ant colonies in forests. Its objective is to determine what factors separate random locations from locations with IFA colonies. Transects have been chosen from within the Tombigbee National Forest (Ackerman Tract) and the John Star Memorial Forest. Transects averaged a kilometer in length and are being considered as one long transect equaling 18.85 kilometers. Along this transect, surveys are being conducted every 500 meters and at every IFA colony. Information on site factors such as soil characteristics, ant community composition (using baits) and canopy-cover, are collected at the survey location. Landscape factors such as landscape composition, landscape configuration and landscape connectivity, will be quantified for each survey location using remotely sensed images and FRAGSTATS (McGarigal 2002) software. Discriminant analysis will be used to identify those factors that distinguish locations with colonies from random locations.

At this time, about half of the transect distance has been covered. The major habitat types surveyed have been early successional pine, mature managed pine and mature hardwood. Twenty-one random points have been surveyed and 6 points with IFA colonies have been found and surveyed. Of the six colonies, 4 were in early successional pine, 2 were in mature managed pine and none were in mature hardwood. IFA were also collected in baits at 3 of the random survey locations. One was in early successional pine and the other 2 were close to the forest edge in a mature hardwood forest and a young dense pine stand. The remaining transect distance will be surveyed in the summer and early fall of 2005.

Part II: Factors effecting the survival and growth of imported fire ant colonies in forests

Part II focuses on the influence of site factors by removing the possible influence of landscape factors. Thirty-six fire ant colonies have been placed in four habitat types (9 colonies per habitat) within the Tombigbee National Forest. The habitats used were mature managed pine, mature hardwood, field and forest edge. The colonies were contained within screened cylinders to allow passage of the worker ants while restricting the movement of the fire ant queen, thereby preventing movement of the colony. The initial population of each colony was estimated using a measure of mound volume that is highly correlated with population size (Macon and Porter 1996). These colonies will be monitored over the summer of 2005 and excavated in the fall 2005, when the final population size will be estimated. Other data to be collected are air and soil temperature (seasonally), ant foraging competitors (mid-summer) and ground and canopy cover (mid summer). Analysis of variance will be used to determine whether there is an effect of habitat type. The influence of soil and air temperature, and canopy and ground cover, will be evaluated using multiple regression. Ant communities in the different habitats, and at locations with and without colonies within those habitats, will be compared using Mantel tests.

Conclusion

The preliminary results from Part I suggest a negative relationship between canopy cover and the occurrence of imported fire ants in forests, something that would be expected considering they are primarily open area species. Openings in canopies have potential impacts on a variety of site factors including sunlight, under-story growth, temperature and arthropod assemblages. Another possible relationship that is not fully understood is the importance of canopy openness as a landscape factor. Do as many potential recruits (inseminated females) reach the floor of a hardwood forest with a closed canopy as would reach the floor of a mature pine forest? By placing fire ant colonies equally in different forest habitats we hope to answer this and other questions that are not sufficiently answered by simple correlation.

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Christmas Tree Farmers' Perceptions and Practices Associated with Imported Fire Ants (*Solenopsis* spp.)

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This project explores three main areas surrounding imported fire ants (IFA) (*Solenopsis* spp.), and the potential habitat found within Christmas tree plantations in the South. The main objectives are as follows:

1) conduct a survey, via questionnaire, of the LA-MS Christmas Tree Farm Association (CTFA) members;

2) determine how the heterogeneity of Christmas tree plantation landscapes relates to the distribution of imported fire ants; and

3) determine if imported fire ant distribution is affected by the use of common cultural practices that can cause fragmentation and/or disturbances.

Only the first objective will be discussed here. The information we presented highlighted results obtained from the survey of the LA-MS CTFA. The questionnaire was developed and patterned after Molnar et al (2003) specifically to:

1) determine problems associated with imported fire ants, as perceived by Christmas tree farmers;

2) determine Christmas tree farmers' level of interest in controlling imported fire ants, and to summarize their general cultural practices; and

3) obtain demographics for Christmas tree growers and their farms in LA and MS.

The questionnaire was mailed to 32 farmers across 17 parishes in Louisiana, and 31 farmers across 22 counties in Mississippi. Forty replies were received out of the 63 sent, representing a 63.5% return rate. Data obtained were analyzed using simple percentage statistics in SAS®.

Of the farmers surveyed, 82.5% felt IFA caused the most harm in the area of potential customer liability, and 37.5% felt IFA encounters with employees was a major problem. The liability results are probably related the type of operation run at each farm. Most farms are choose-and-cut operations, where the customer may choose and cut his/her own tree, and encounter IFA. Additional weight may be given to liability issues because many of the farmers host special events for local public school children. These events, such as Easter egg hunts in the spring, and pumpkin patch days in the fall, could put the children at risk of being stung.

Minor damage to trees was indicated by 47.5% of the respondents, while 30% reported no damage to trees. We also found that 37.5% reported no customer losses due to IFA presence, while 45% were unsure and 15% thought minor losses were possible. It is interesting to note that the areas that reported highest customer liability and minor customer losses were located in the southern-most regions of Mississippi and Louisiana, where red imported fire ants (*Solenopsis invicta* Buren) (RIFA) are most prevalent.

In the area of landowner interest, we found that 62.5% were very interested in controlling IFA, 27.5% had only slight interest, and 7.5% had no interest. The "no interest" responses were solely from extreme northern counties in Mississippi, where RIFA are less abundant, and where black imported fire ants (*Solenopsis richteri* Forel) are more prevalent. Thirty-five percent of the respondents included control measures for IFA in their management plans.

The questionnaire also covered general cultural practices, such as mowing and pesticide practices. This information was important in determining general landscape disturbance events, because fire ants have been shown to live in, and even prefer, extremely disturbed habitats. We found that over 80% of growers surveyed regularly mow and/or bush-hog and apply herbicides for weed control. They also apply insecticides to control fire ants. Additionally, all respondents use various combinations of fertilization, herbicide, pesticide, mowing and shearing, as regular farming practices. Within the LA-MS Christmas Tree Growers Association, all farmers grow leyland cypress (*Cupressus cyparis* X *leylandii*), while seventy percent grow virginia pine (*Pinus virginiana*), and carolina sapphire (*Cupressus arizonica*). The only other major tree species grown is the eastern red cedar (*Juniperus virginiana*), and it is slowly being phased out do to its lack of popularity with consumers.

In determining farm demographics and landowner descriptions, we discovered that approximately half of the farms were less than 10 acres in size, and have been in operation over 20 years. Approximately 98% of the farms are choose and cut operations, and about 50% of the farms were started to provide supplementary income to retired couples between the ages of 50-70 years of age. Interestingly, over half of the respondents were college graduates or have had some college education.

In summary, respondents identified potential liability as the major IFA problem, followed by potential employee encounters. These results are attributed to the farms choose-and cut operations, and involvement with local school children. Basic control measures employed by respondents were applying insecticides, and mowing regularly. Over 60% of respondents were highly interested in learning new measures to control IFA better. We also observed a possible link between interest in controlling IFA, and the southern/northern location of the farm. Finally, we found that the majority of farmers are retired couples who have had a college education, and are trying to supplement their income.

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Exclusion of RIFA to Prevent the Predation on the Eastern Bluebird

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Bluebird nest boxes have been monitored in the Midlands of South Carolina by the Department of Natural resources since 2000. The total number of nest boxes has ranged from a low of 72 boxes in 2001 to a high of 246 in 2004. Fire ant predation of the chicks has ranged from 3% to 16% during the study. In 2004 a baffle was used to attempt to exclude the fire ants from the nest boxes. The baffle consisted of an inverted 1 liter soda bottle cut as seen in included photo. The soda bottle was coated with fluon or tanglefoot. In 2004 a total of 246 nest boxes were used 49 of those boxes were located at Sandhill Research and Education Center (REC). A total of 61 nests were predated, 14 of the predated boxes were located at Sandhill REC. Twenty-three (23) of the predations were attributed to fire ants, zero (0) fire ant predations occurred on Sandhill REC where the baffle was used.

Year	Total Boxes	Total Predation	RIFA Predation
2000	139	21	5
2001	72	16	12
2002	170	33	9
2003	214	40	13
2004	246	61	23

Up-regulation of a transferrin gene in response to fungal infection in *Solenopsis invicta*

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The cDNA of a gene with significant homology to insect transferrins was identified and sequenced from *Solenopsis invicta*. Translation of the open reading frame yielded a protein with a molecular mass of 77.3 kDa and p*I* value of 5.66, characteristics consistent with transferrin proteins. Quantitative PCR was used to examine the expression of the *S. invicta* transferrin. Among different developmental stages selected, early pupae exhibited the highest expression level, with significantly lower expression levels in late larvae, queens, and workers. Expression was induced in worker ants exposed to varying doses of *Beauveria bassiana* conidia. Killed conidia did not elicit an inductive response. Worker ants exposed to juvenile hormone exhibited a significantly lower relative expression of *S. invicta* transferrin compared with an untreated control. However, expression of *S. invicta* transferrin was not significantly different in ants exposed to 20-hydroxyecdysone. Genes, like the *S. invicta* transferrin, responding to pathogen attack may provide a unique approach for the discovery of microbial control organisms from fire ants.

Prevalence of *Thelohania solenopsae* infected *Solenopsis invicta* newly mated queens within areas of differing social form distributions

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Abstract
Newly mated queens (NMQs) originating from monogyne red imported fire ant (*Solenopsis invicta*) colonies and following a mating flight, initiate new colonies by sealing themselves in a nuptial chamber and using stored food reserves to rear their first workers (claustral colony foundation). This method of colony founding is rarely successful for polygyne-derived NMQs, whose low weight critically limits the number of first workers produced. However, this observation may be confounded by the parasitic microsporidium, *Thelohania solenopsae*, thus far predominately found in association with polygyne colonies. This microsporidium reduces fat reserves within infected queens and may explain why polygyne NMQs are unable to found colonies claustrally. We collected NMQs following mating flights in Gainesville and Ocala, Florida. Newly mated queens were sorted by weight class, then checked for insemination and *T. solenopsae* infection. Insemination levels were greater than 90% for all weight classes at both collection sites and were not related to infection. Infection levels were lower in Gainesville than Ocala, averaging 1.67% and 14.14%, respectively. Polygyne-derived NMQs collected in Ocala, defined here as weighing ≤ 12 mg, had the highest infection levels, 25.37% (17/67) in 2003 and 21.43% (6/28) in 2004. Therefore, infection by *T. solenopsae* cannot be completely responsible for the inability of polygyne NMQs to claustrally establish colonies. This work highlights the need to consider potential parasites and pathogens when collecting fire ant colonies for research.

Status of *Thelohania solenopsae* in the Red Imported Fire Ant population in Oklahoma: Implications from a 2-year study

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Abstract

The absence of natural enemies is one of the key factors that enabled Red Imported Fire Ant, *Solenopsis invicta* to flourish and spread in the southern United States of America. *Thelohania solenopsae*, a microsporidian parasite and *Pseudacteon tricuspis*, a phorid fly are two major natural enemies of fire ants in South America. Recent investigations have focused on *T. solenopsae* as a potential biocontrol agent for S. *invicta*. In this study, we have documented the distribution patterns of *T. solenopsae* in the populations of *S. invicta*, in southeastern Oklahoma. Fire samples were collected along the highways in southeastern Oklahoma in 2003 and 2004. The presence/absence of the parasite in the fire ant population indicated the movement of the infection in fire ants along with the movement of the infected queens in the state. In 2003, eighty-two samples were collected from ten counties, of which only 6 were infected. Most of these infected samples were from Bryan Co., OK. Eighteen counties were sampled in 2004, of which three counties never had fire ants. *T. solenopsae* infection was widespread in Bryan, Carter, Choctaw, Johnston and Marshall Counties. Moreover, the infected fire ant population had moved 10 miles further east into Choctaw Co., as compared to the status in 2003. This is an ideal system to study the mechanisms involved in the stabilization of the parasite in the fire ants. In addition, this is also an interesting model to study the strategies that the fire ants may employ to encounter parasitization. Our future investigation will continue to focus on the spread of *T. solenopsae* among the fire ant population in Oklahoma and the response of the fire ants towards parasitization.

Introduction

Since the accidental introduction in the 1930s, *Solenopsis invicta* has emerged as one of the major invasive insect species in the United States. It infests over 112 million hectares in the United States (Lofgren, 1986) and impacts the environment in numerous ways. *S. invicta* is more abundant in the United States than in its natural habitats in South America (Porter *et al.,* 1992), probably due to lack of natural enemies in the introduced habitats. Several workers have recently begun to focus on using sustainable control methods, which mainly includes biological control agents. *Thelohania solenopsae*, a microsporidian obligate intracellular pathogen has been an effective natural enemy of the fire ants in Argentina (Briano *et al.,* 1995). *T. solenopsae* declines the egg production, queen weight and survivability of queens and workers of *S. invicta* (Oi and Williams, 2002). Cook (2002) observed infected and uninfected colonies and found that the colonies infected with *T. solenopsae* had lesser mound volume than the uninfected colonies. Also, the colony densities were positively correlated to percentage of infection.

In 1998 and 2000, spores of *T. solenopsae* were released in Bryan Co., OK, and Carter Co., OK, respectively. However, the status of the pathogen remained uncertain. For the first time in Oklahoma, fire ants collected in October 2002 from elsewhere in Bryan Co., tested positive for the microsporidian pathogen as confirmed by Polymerase Chain Reaction and modified Trichrome staining methods. Our preliminary analyses indicated the presence of the pathogen in fire ants collected from Bryan Co., but not from those collected from the southeastern county – McCurtain. Hence, a monitoring program was initiated wherein, the fire ants were collected using hotdog baits along highways in

Oklahoma, in summer of 2003 and 2004. The salient findings from this 2-year study are described in this article.

Materials and Methods

Fire Ant samples were collected along the state highways of southern Oklahoma in July-August 2003 and 2004. Hot dog baits were placed on either sides of the highway, every five miles. The baits were collected after 45-60 minutes and placed on ice. Each sample was identified to species, in the laboratory and *S. invicta* samples were frozen until analyzed for the presence of *T. solenopsae.* The samples were tested for the presence of *T. solenopsae* spores using PCR and modified trichrome staining.

Polymerase Chain Reaction:

Fifteen to thirty ants were ground with 500µl TBS using a disposable pestle. To this homogenate, 0.1mm glass beads were added up to three-quarters of the tube and beaten at maximum speed for 15 seconds, in a bead beater. The tubes were immediately transferred and kept in 95°C water bath for 5 min. The samples were spun for 10sec at 18000g and the supernatant containing genomic DNA of *T. solenopsae* was collected. 2µl of the total DNA was added to the mixture of *T. solenopsae* specific primers - Msp1a and Msp4b (1µl each) and Ready-to-go- PCR beads. This mixture was made up to 25µl with sterile distilled water and the PCR reactions were set following the protocol of Snowden *et al.*, 2002. PCR products were separated on 2% agarose gels and visualized by Ethidium bromide staining. For all experiments, positive (*T. solenopsae* DNA) and negative (PCR reaction mixture without total DNA) controls were also run along with treatments.

Trichrome staining:

Fifteen to thirty ants were ground with 500µl TBS using a disposable pestle. About 15µl of the ground solution is placed on labeled glass slide and air-dried. The slides are stained using the modified Trichrome staining protocol of Kokoskin *et al.* (1994). The slides were observed under a light microscope at 400x and 1000x magnifications for the spores of the pathogen (Fig 1a&b).

Results and Discussion

In the 2003 survey, ten counties were sampled. *S. invicta* was commonly found in southern counties and rarely in the northern and northeastern counties. Six of the eighty-two fire ant samples were infected with *T. solenopsae*. Of these six, five were from Bryan Co., and the other was from Choctaw Co. Infected populations of *S. invicta* was found up to 35 miles east from Durant, Bryan Co. In 2004, the survey was expanded to eighteen counties. Fire ants were not found in the baits of three of the 18 counties, and rarely found in Le Flore and Pushmataha counties. Infected populations of *S. invicta* were highly prevalent in Bryan, Carter, Choctaw, Johnston and Marshall counties. The microsporidian infection had spread at least further 10 miles east into Choctaw Co., in 2004, as compared to the status in 2003.

Even though the spread and establishment of *S. invicta* population is alarming in Oklahoma, the parallel spread of the microsporidian pathogen, *T. solenopsae* amidst the fire ant population may help in the long-term management. Stabilization of *T. solenopsae* in the fire ant populations in Oklahoma will largely depend on the spread, establishment and survival of infect, newly-mated queens. High turnover of infected and healthy queens initiating colonies in the periphery of established regions, especially after every winter will be crucial in sustaining the microsporidian in the fire ants. The status of *T. solenopsae* in the RIFA population of Oklahoma is an ideal model to study the interactions between the two species and the strategies they may adopt for survival.

Acknowledgments

We thank Forrest Mitchell for training VK to analyze fire ant samples using PCR and modified trichrome staining method.

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Table 1. Patterns of *T. solenopsae* infections in *S. invicta* population in 2002.

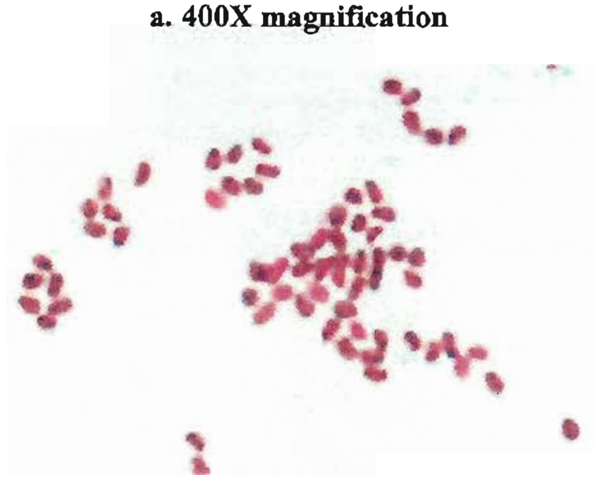
Sites	Total number of plots	Number of plots sampled	Number of plots analyzed	Number and percent of infected plots
Adam's Ranch, Bryan County	19	16	14	12 (85.7%)
Bowles' Ranch, Bryan County	22	19	19	17 (89.5%)
McCoy's Ranch, McCurtain County	20	19	14	0

Table 2. Patterns of *T. solenopsae* infections in *S. invicta* population in 2003.

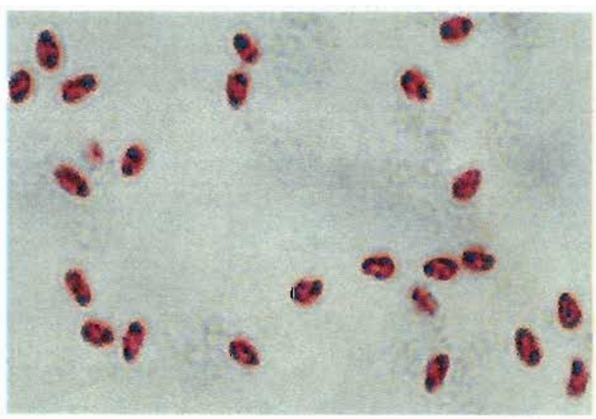
Sites	Total number of plots	Number of plots sampled		Number of plots analyzed		Number and percent of infected plots	
		Spring	Fall	Spring	Fall	Spring	Fall
Adam's Ranch, Bryan County	19	17	17	12	17	3 (30%)	12 (70.6%)
Bowles' Ranch, Bryan County	22	19	17	15	17	5 (33.3%)	10 (58.8%)
McCoy's Ranch, McCurtain County	20	17	20	14	20	0	0

Fig 1. Photomicrograph of binucleate free spores detected from RIFA samples

a. 400X magnification



b. 1000X magnification



Factors Affecting Longevity of the Phorid fly *Pseudacteon tricuspis*: Effects of Sugar availability, Temperature, Mating, and Size

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The phorid fly *Pseudacteon tricuspis* is one of two species of phorid flies that have been released for biological control of red imported fire ant, *Solenopis invicta* in many parts of the southern United States. Several aspects of the biology of phorid flies and their potential to control imported *Solenopsis* fire ants have been investigated (reviewed by Porter 1998, Morrison, 2000), and attempts are currently being made to monitor and evaluate the establishment and impact of phorid flies at various release sites. However, several important questions remain unanswered regarding the biology and ecology of phorid flies. For instance, little is known about the nutritional ecology of *Pseudacteon* spp. under laboratory or field conditions, and the effects of environmental and physiological factors on lifespan and reproduction have not been examined.

We report here the effects of sugar feeding, temperature, mating, and body size on longevity of female and male *P. tricuspis*. Sugar availability and temperature were the two major factors influencing longevity. In general, sugar feeding increased lifespan by a factor of 2-3. Longevity of *P. tricuspis* was inversely related to temperature, and the greatest longevity (~15 days) was recorded for sugar fed flies kept at 20°C (the lowest temperature evaluated in this study). Gender and mating did not significantly influence longevity. Gender, however, had a significant effect on wing length with females being larger than males. These results suggest that provision of supplemental sugar sources and suitable microclimate near its release sites may enhance the success of *P. tricuspis* as a biological control agent for imported fire ants.

Materials and Methods

Adult *Pseudacteon tricuspis* used in this study were reared on workers of red imported fire ants, *Solenopsis invicta,* at the fire ant rearing facility of the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA. Parasitized fire ant worker heads were received in batches and kept in a plastic jar (25 × 13 cm) with a lid until emergence. Emerging flies did not have access to food or water in the jar. Newly emerged flies were removed promptly with an aspirator and sexed immediately under a dissecting microscope.

The experiment simultaneously tested the effects and interactions of diet (sugar fed vs. sugar starved), temperature (20, 25, 28, and 33 °C), mating status (mated vs. unmated) on longevity of female and male *P. tricuspis*. The various combination of these factors resulted in a total of 16 treatment combinations for each gender. The methodology and protocols have been described in detail by Chen et al. (2005). Newly emerged flies were placed in groups of 2 individuals either of the same gender (unmated treatments) or of opposite gender (mated treatments) in a 6-cm diameter plastic Petri dish. Water was provided in all treatments. For the treatments involving sugar feeding, 25% sucrose solution was smeared on the inside of the Petri dish cover every three days. Petri dishes were kept in separate growth chambers at 20, 25, 28, or

33°C with a 14:10 (L:D) photoperiod and 75±5% r.h. At least 16 flies of each gender were tested for each treatment combination. Petri-dishes were checked once daily for dead flies, which were promptly removed from the dishes. The effect of fly size (wing length) on longevity of *P. tricuspis* was evaluated by measuring one forewing from each dead fly to the nearest 0.05 mm. We tested the effects and interactions of diet (sugar availability), temperature, gender, mating status, and wing length (as a measure of size) on survivorship using proportional hazard modeling, a nonparametric analysis designed to evaluate for effects of multiple factors on survivorship (SAS Institute 1998). Longevity data for each gender was analyzed separately by using ANOVA followed by the Tukey-Kramer HSD test for multiple comparisons of means at $P < 0.05$ (SAS Institute 1998).

Results and Discussion

Statistical analysis revealed significant effects of diet, temperature, and wing length on longevity of *P. tricuspis*. Diet significantly influenced longevity with sugar fed flies living significantly longer than sugar starved flies ($\chi^2 = 159.51$, df = 1, $P < 0.00001$). Similarly, temperature had a significant effect on survivorship ($\chi^2 = 188.96$, df = 3, $P < 0.00001$): longevity was greater at lower temperatures than that at higher temperatures (Table 1). Longevity was also significantly influenced by wing length ($\chi^2 = 4.78$, df = 1, $P = 0.03$). However, the effects of gender ($\chi^2 = 3.28$, df = 1, $P = 0.07$) and mating ($\chi^2 = 0.44$, df = 1, $P = 0.51$) were not significant, suggesting that adult longevity did not vary as a result of gender or mating status. Also, there were no significant interactions among any of the variables. Proportional hazard analysis shows that wing length was significantly affected only by gender ($\chi^2 = 126.62$, df = 1, $P < 0.0001$), with males having a significantly shorter wing length (mean ± SD = 1.04 ± 0.09 mm; N = 256) than females (mean ± SD = 1.14 ± 0.08 mm; N = 256) *P. tricuspis* ($t_{510} = 12.90$; $P < 0.001$).

These results showed that sugar availability and temperature were the two main factors affecting adult lifespan. Sugar fed female and male *P. tricuspis* had significantly greater longevity than their sugar starved counterparts. In general, sugar feeding increased phorid fly lifespan by a factor of 2-3 relative to sugar starved flies, irrespective of the temperature. These results are in agreement with previous reports of the positive effect of sugar feeding on longevity of several hymenopteran parasitoids (Heimpel et al. 1997, Olson et al. 2000, Fadamiro and Heimpel 2001, Wäckers 2001). The significant effect of sugar feeding on lifespan suggests that *P. tricuspis* may benefit from sugar availability in the field. In the field, parasitoids can potentially obtain sugar from different sources including floral or extra-floral nectar and homopteran honeydew (Jervis et al. 1996, Heimpel and Jervis 2005). Future studies on the nutritional ecology and foraging behavior of *P. tricuspis* will address its possible utilization of sugar sources in the field and provide insights into the effects of sugar availability on the fecundity and performance of phorid flies as a biological control agent of imported fire ants in southern United States.

Temperature also had a significant effect on longevity of *P. tricuspis* in the current study with longevity being inversely related to temperature. The greatest mean longevity (~15 days) was recorded for sugar fed flies kept at 20°C. Longevity was consistently greater at the lower temperatures (20°C and 25°C) than that at the higher temperatures (28°C and 33°C), irrespective of the diet. Increasing the temperature from 20°C to 33°C resulted in approximately 3-4-fold reduction in longevity. Similar results have been reported for other insects, including several parasitoids (Dyer and Landis 1996, McDougall and Mills 1997). In Alabama and other parts of the southern United States where *P. tricuspis* has been released for biological control of imported fire ant, *S. invicta*, average daily temperatures in the summer are usually high and sometimes in excess of 30°C, and relative humidity is frequently above 75%. Our results showing that adult *P. tricuspis* is sensitive to high temperatures coupled with the fact the parasitoid may likely experience more severe heat in the field from direct solar radiation, suggest that hot summer temperatures may potentially impact its establishment and performance in the southern United States. However, several habitats such as woodlots and other non-crop habitats near the release sites of *P. tricuspis* could potentially provide cool or moderate microclimates for the parasitoid thereby limiting its exposure to severe temperatures. Field studies on the behavioral ecology, dispersal ability, and habitat utilization by *P. tricuspis* will provide insights into how the parasitoid avoids stressful environmental conditions.

In conclusion, our data demonstrated significant effects of sugar availability and temperature on lifespan of *P. tricuspis*. These results suggest that availability of adult food sources and suitable microclimate are important factors that could potentially influence the survival, establishment, distribution, and overall impact of *P. tricuspis* released in different parts of southern United States for biological control of imported fire ants. Provision of supplemental sugar sources and suitable microclimate near release sites may likely enhance the success of phorid flies as a biological control agent.

Acknowledgements

We thank Debbie Roberts (USDA APHIS PPQ CPHST Laboratory, Gainesville, FL, USA) for the supply of adult *P. tricuspis* for this study. This research was funded partly by the Alabama Fire Ant Management Program grant to HYF, and by the Alabama Agricultural Experiment Station.

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Table 1. Effects of sugar feeding and temperature on longevity (days ± SE) of female and male *P. tricuspis*.

Temperature (°C)	Female		Male	
	Sugar starved	Sugar fed	Sugar starved	Sugar fed
20	7.3 ± 0.42 a (b)	14.8 ± 1.11 a (a)	6.1 ± 0.44 a (b)	14.5 ± 1.48 a (a)
25	3.3 ± 0.19 b (b)	10.0 ± 0.75 b (a)	2.8 ± 0.19 b (b)	9.9 ± 1.12 b (a)
28	2.3± 0.14 c (b)	5.3 ± 0.61 c (a)	2.3 ± 0.16 b (b)	5.7 ± 0.70 c (a)
33	2.1 ± 0.13 c (b)	4.1 ± 0.30 c (a)	2.0 ± 0.08 b (b)	4.5 ± 0.41 c (a)

Means within the same column having different letters are significant ($P < 0.05$, Tukey HSD test). Similarly, means across the same row having different letters indicated in parentheses are significant ($P < 0.05$, Tukey HSD test).

Phorids Can Be Released in Cold Weather: A History of Alabama Releases

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Abstract

Two species of phorid fly, *Pseudacteon tricuspis* and *Pseudacteon curvatus*, have been successfully released in Alabama. *Pseudacteon tricuspis* was released in populations of the red imported fire ant, *Solenopsis invicta*, and *P. curvatus* was released in populations of the hybrid fire ant (*S. invicta* x *Solenopsis richteri*). Twelve releases have been conducted and field-reared phorid flies have been recovered at eleven of the release sites (see map). All but two releases were conducted by our lab. The releases of *P. curvatus* in Lauderdale and Madison counties were conducted by Ken Ward and Rufina Ward of The Department of Plant and Soil Science at Alabama A&M University in Normal, AL.

Pseudacteon curvatus were established by shipping field collected ants to the phorid rearing facility in Gainesville, FL as described by Graham et al. 2003 or Starkville, MS as described by Vogt and Street 2003. Ants were exposed to phorids in the rearing facility and returned to the field approximately one week later.

Fig. 1 Phorid Emergence Container

Pseudacteon tricuspis were established by releasing newly emerged flies at the site. Flies are released no later than the day after emergence. At the Macon County release site, mounds were disturbed and 60 phorids were released from a container placed near the mound (Fig. 1). Mounds were disturbed on a regular basis for a two hour period to keep the ants active.

At the remaining sites, a plastic under-bed storage container was used as an attack box. The box was modified by cutting holes into the lid for ventilation, covering the openings with mesh (NoSeeum netting, Balson Hercules, Providence, RI) and drilling a small hole into the lid to introduce the phorid flies. A single mound was excavated and the ants and soil were placed into each box. A lid was placed on the box and 60 *P. tricuspis* were introduced through a hole in the lid. After two to three minutes, the lid was removed. Phorids continued to attack for approximately two hours if the ants were disturbed often by stirring the soil in the box or shaking the box. This agitation prevented the ants from hiding under the soil or in corners to escape attack. The ants were returned to the mound from which they were originally removed (Fig. 2).

Fig.2 Field release using attack boxes

Temperatures during most releases were 21° C or higher and the phorids were active at these temperatures. On days when temperatures were below 21° C, flies were inactive and did

not attack. Flies were not harvested for release on cold days or on rainy days, but were held overnight and released the following day.

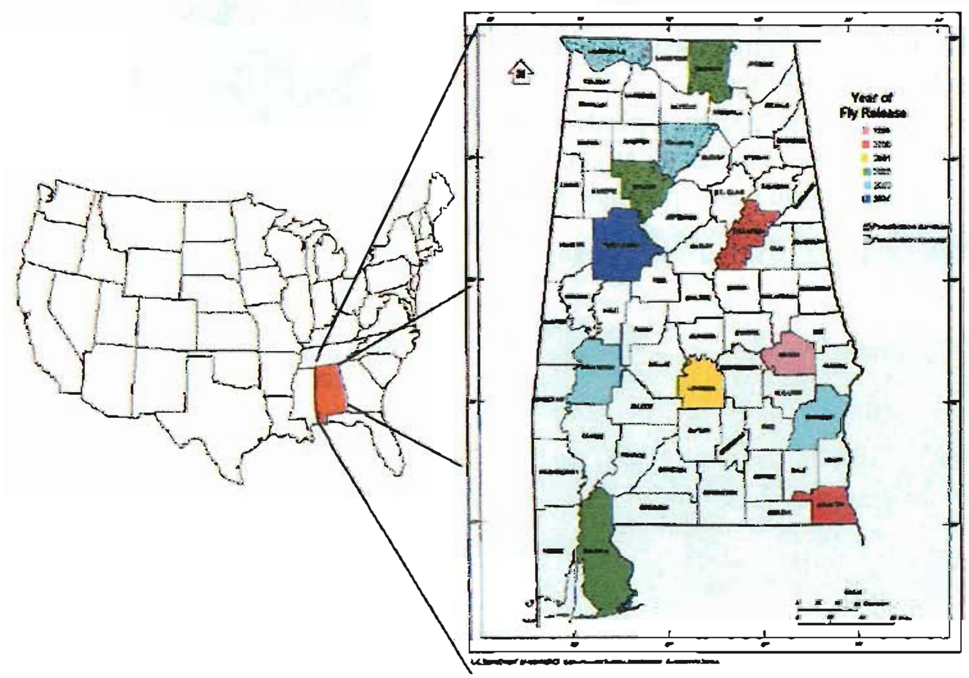
During the 2004 release in Tuscaloosa County, temperatures remained below 21 ° C or rains occurred on four consecutive days. Since the flies were not active in these field conditions, we modified our release protocol and conducted the release indoors (Fig. 3). The attack boxes were set up inside the office building where the phorid pupae and newly emerged flies were maintained at 29 ° C. Flies were placed into the attack boxes and the lids were kept in place.


Fig. 3 Phorid release indoors

The ants were disturbed only by shaking the boxes. The presence of brood tended to keep the ants more active, allowing phorids more opportunity to attack. The flies were allowed to attack in the covered boxes for the regular two hour period. Ants and phorids were taken back out to the release site where the phorids were released and the ants were returned to the mound from which they were originally removed.

The success or failure of this release is probably not dependent on the change in release protocol during the cold or rainy days. Over 6,300 flies were released over a two week period. This is the largest number of flies we have ever released at a site. However, 3,424 phorid flies emerged on the four cold and rainy days and their chances of success were enhanced by the indoor release protocol.

Approximately six weeks after the first flies were released on April 7, phorids (3-6 per mound) were active at approximately 75% of the mounds checked at the site. This is the largest population of phorids recovered immediately following a release to date.



Map of phorid releases in Alabama

Host Location Behavior of *Pseudacteon curvatus* in Alabama

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Imported fire ants are an accidentally introduced invasive species of ant that is native to South America. The black imported fire ant, *Solenopsis richteri*, entered the port of Mobile around 1918 and the red imported fire ant, *Solenopsis invicta*, entered later in the 1930's. One reason fire ants have been so successful and have spread so rapidly is their natural enemies were left behind in South America (Jouvenaz 1990). In an attempt to tip the ecological balance in favor of native ants and to reduce imported fire ant abundance, a parasitoid has been introduced into fire ant populations in the United States (Porter 1998; Graham *et al.* 2003; Vogt *et al.* 2003; Williams et al. 2003; Porter *et al.* 2004). The parasitoid is a phorid fly that attacks *Solenopsis* fire ants. There are over twenty species of phorid fly in South America. In Alabama, two species of phorid fly have been released: *Pseudacteon tricuspis* and *Pseudacteon curvatus*.

Pseudacteon tricuspis has been released in seven counties in Alabama. *Pseudacteon curvatus* has been released in five counties in Alabama. Morrison and King (2004) examined the host location behavior of *P. tricuspis*. This study is designed to help answer some questions about the host location behavior of *P. curvatus*.

Our experiments on *P. curvatus* were modeled after the methods Morrison and King (2004) used in their experiments on *P. tricuspis*. The study was located at the *P. curvatus* release site in Talladega County, Alabama. The site is an area that was at one time a clear-cut area turned pasture with grazing cattle, but is now a fallow field used for quail, dove, and turkey hunting. The site has monogyne colonies of imported fire ants.

We first determined the diurnal pattern for *P. curvatus* in Alabama. We wanted to find the times of day when *P. curvatus* are most active and available for use in field experiments to facilitate efficiency in the field.

Ants used in the study were collected from the vicinity of the original release site just prior to field data collection. Ants from four mounds were returned to the lab and were separated from the soil. Each colony was placed into an individual 52 x 40 x 13 cm plastic tray lined with Fluon® (Asahi Glass Ltd., Chadds Ford, PA). The four trays of ants were placed in shady areas of the release site about 8 m apart. To induce ant pheromone release, the ants were agitated by shaking the trays. Thirty minutes after agitation, phorid flies were aspirated out of their tray using a double chambered aspirator unit until no flies could be observed in the tray. The flies were then transferred from the aspirator to a 14 x 14 x 7 cm plastic holding container via a hole in the lid. Carbon dioxide was introduced into the holding container to induce fly knock down. Upon knock down, the lid was removed from the container and flies were counted. After fly count, the container was placed in the shade to allow for fly recovery. The tray was shaken again and collection moved to the next box. Collection started about 2 ½ hours following sunrise (HFS). Data were collected every 30 minutes until flies ceased coming to the trays, about 12-13 hours following sunrise or about dusk.

In 2002, 1,247 flies were collected in August. Activity decreased in September. In 2003, fly collection began in June and numbers were relatively low. Fly numbers peaked in July and August and then decreased again in September. In 2004, collection began in May when fly numbers were low. By July 2004, fly numbers had increased. Data in figure 1 suggest a cyclic wave of fly activity for *P. curvatus* in Alabama with populations peaking in August.

Figure 2 is a graph of the mean number of *P. curvatus* collected each sample period. Moderate fly activity occurs from four to twelve hours following sunrise. *Pseudacteon curvatus* has a bimodal activity pattern with a minor peak of activity about five HFS and a major peak in activity is seen 10-11 HFS.

For experiments 2-5, some terms should be defined. A "bait" consists of a 4 g section of Gwaltney chicken hot dog placed on a 5 x 5 cm laminated card. The bait cards are spaced 5 m apart on one or more transects. When a "disturbance" was applied, a crater about 8" x 8" was dug into the mound using a small shovel. When a "non-nestmate disturbance" (NNM) was applied, a cup with a predetermined amount of ants from a laboratory colony were transported to the field and applied to either a bait card and/or a mound. When a "shock" treatment was applied, it was administered using a modified electric cattle prod similar to one developed by Charles Barr, Texas A&M University.

For the second experiment, we tested whether *P. curvatus* are more attracted to workers at a disturbance or to workers at a food source. We hypothesize that flies will be more attracted to the workers at the disturbed mounds. This experiment was a two part experiment conducted at different times of the day to prevent exposure of flies to two different stimuli. For the first part, 30 baits were placed on a transect 5 m apart. After 20 minutes, baits were monitored for fly presence at 20 minute intervals for one hour. For the second part of the experiment, 30 mounds were disturbed by digging a crater as previously described. After 10 minutes, mounds were monitored for fly presence at 10 minute intervals for 30 minutes.

There was a significantly higher number of flies present at the disturbance with 25 of 30 mounds having flies present over the 30 minute monitoring period as opposed to the baits that had only 10 of 30 baits with flies present after one hour (fig. 3). The foraging ants attracted a total of 34 flies over the one hour monitoring period while the ants at the disturbed mounds attracted 443 flies over the 30 minute monitoring period (fig. 4).

At each collection interval, the baits had relatively low fly numbers. However, at the disturbed mounds, flies were found at a higher density at all times (fig. 5).

Experiment 3 tested whether flies are more attracted to workers that are simply foraging or to workers that are competing at a food source. Our hypothesis is that the flies will be more attracted to the workers competing at a food source. For this experiment, the baits were placed inside 100 x 15 mm Petri dishes. The dishes had a small hole drilled into the side to allow for a foraging pathway and the dish was also lined with Fluon® to prevent the hasty escape of NNM when they were added to the foragers. Thirty baits were placed in Petri dishes lined with Fluon® on a transect 5 m apart. After one hour of recruitment time, every other bait on the transect received a 200 NNM by weight treatment. Baits were monitored for fly presence at five minute intervals for 20 minutes.

At five minutes, there was no significant difference between baits that had a NNM treatment and ones that didn't. However, at 10, 15, and 20 minutes, we do see a significant difference between baits that have a conspecific interaction and baits that only have foragers (fig. 6). Since recruitment to bait stations was low and only a small number could be used in analysis, the experiment will be repeated next year to obtain more replicates.

The next experiment tested whether or not increasing the interaction at a food resource will affect the number of flies attracted. Our hypothesis is that increasing the number of NNM will increase the number of flies attracted. A total of 30 baits were placed on six transects 5 m apart. Treatments of five, 25, 50, 100, and 250 NNM were randomly assigned to six replicates in a randomized complete block design. Fly presence was monitored at five minute intervals for 30 minutes, then at 10 minutes intervals for an additional 30 minutes for a total monitor time of one hour.

Figure 7 is a graph of the total number of flies that were observed over the entire one hour sampling period. There were no significant differences between the five, 25, 50, and 250 NNM treatments. However, there was a significant difference between the 100 NNM treatment and the 25 and 50 NNM treatments.

Experiment 5 tested whether flies are more attracted to workers at colony disturbances with interaction or to workers at colony disturbances without interaction and then, are they differently attracted to the interactions. Fifteen mounds were disturbed. To the first five mounds, no additional treatment was added. To the next five mounds, a 15 second shock was administered via the modified cattle prod. To the last five mounds, a 300 NNM by weight treatment was administered. All 15 mounds were monitored at 10 minute intervals for 30 minutes.

There were no significant differences between the three treatments at 10 minutes (fig.8). However, at 20 and 30 minutes, there are significantly more flies at the shock treatment than the disturbance treatment. Over the total 30 minute period, there was no significant difference between fly numbers at the shock and NNM treatments, but there were significantly more flies at these two treatments than at the disturbance alone.

In summary, we found that *P. curvatus* in Alabama are more attracted to fire ants at a colony disturbance (whether it is a mound disturbance or a NNM disturbance) rather than to fire ants at a food source. We also found that while phorids are attracted to NNM disturbances, attraction was not enhanced by increasing the number of NNM. Lastly, we found that *P. curvatus* are differently attracted to colony disturbances with and without interactions. They are least attracted to a mound disturbance alone. They are more attracted to a colony disturbance where a shock has been applied to the fire ants as opposed to a disturbance where there is a conspecific interaction but the difference is not significant.

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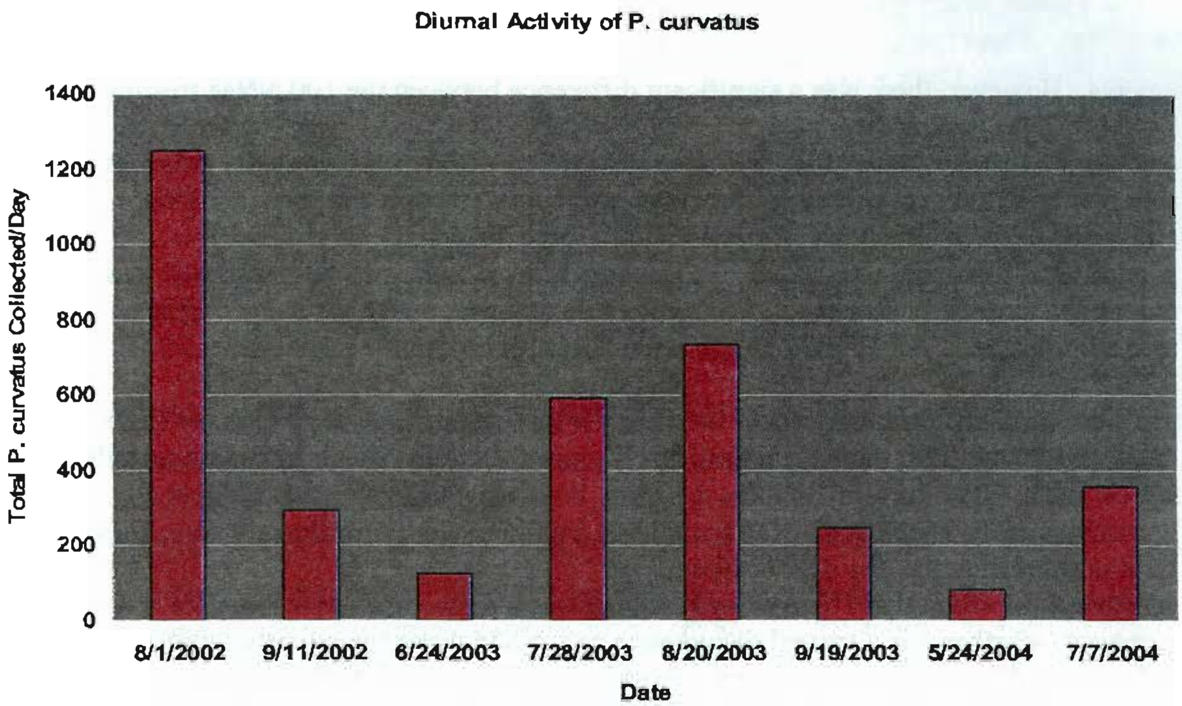


Figure 1.

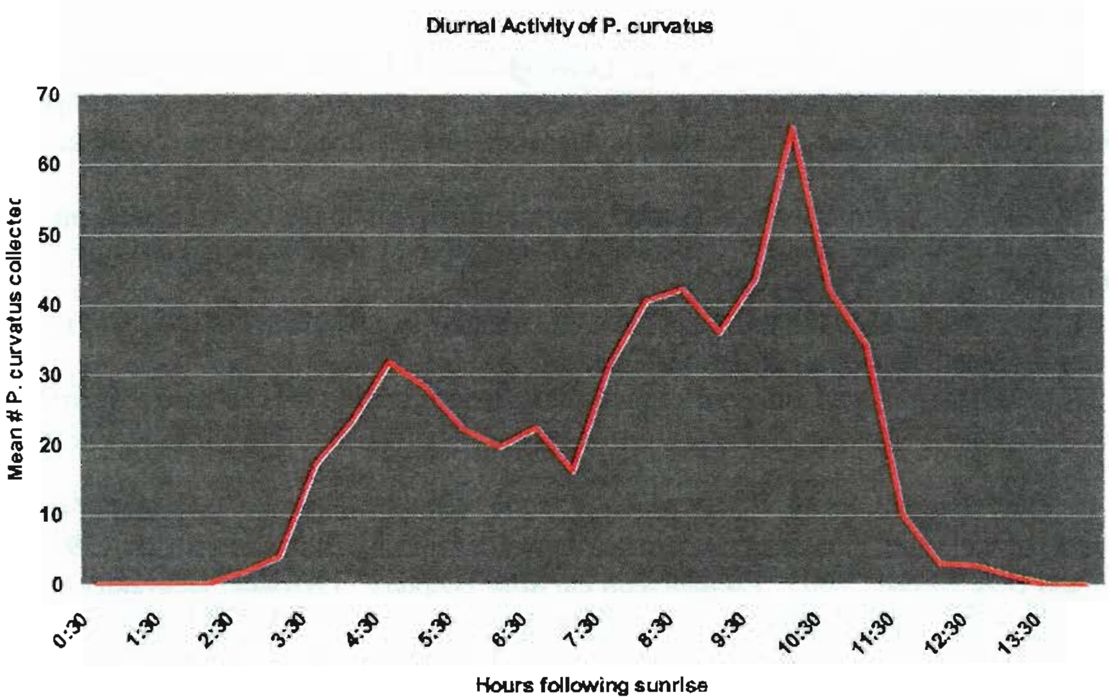


Figure 2.

Fly Presence vs. Fly Absence

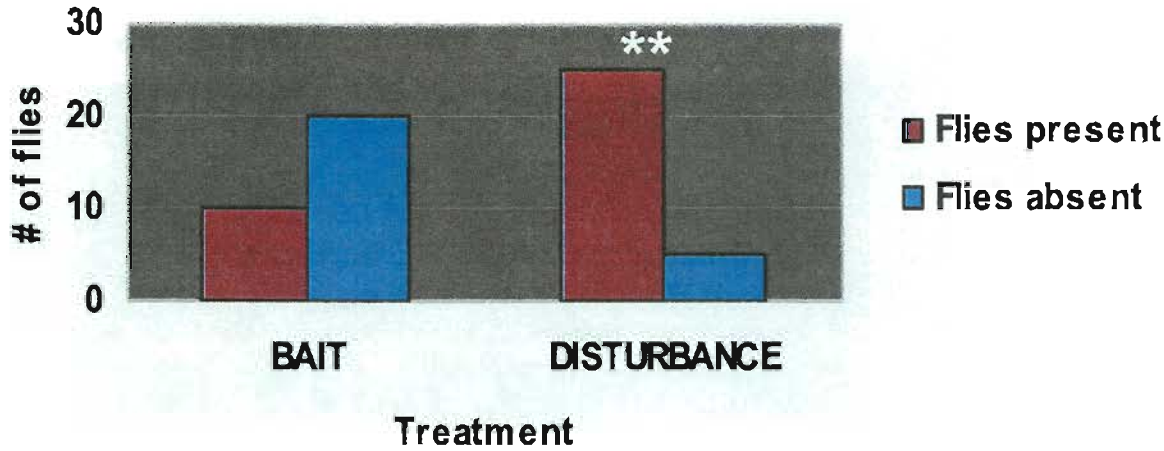
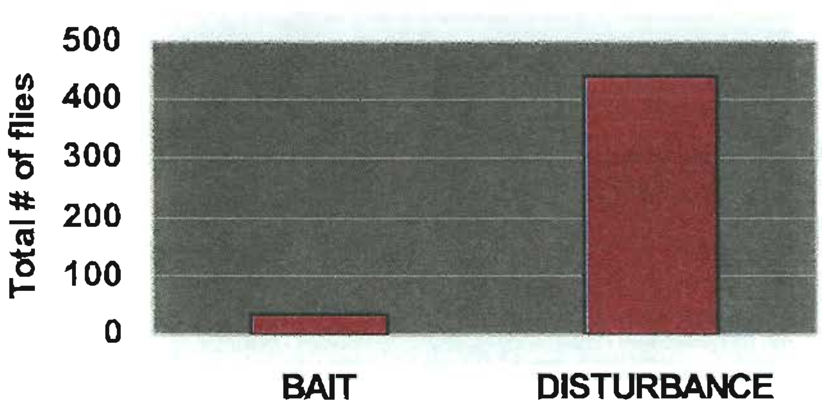


Figure 3.

Total Fly Number



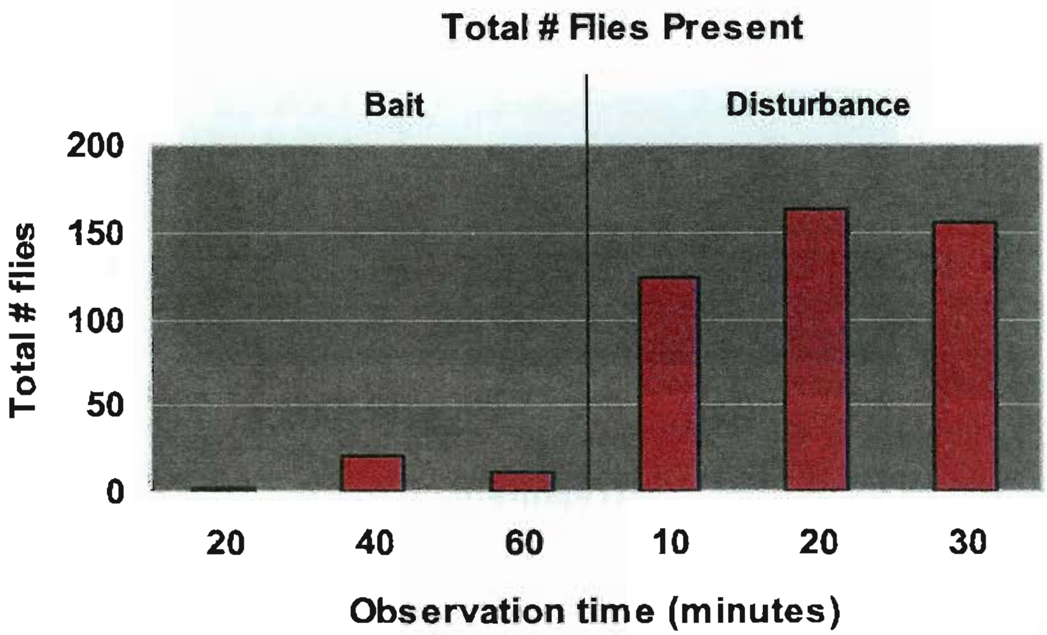


Figure 5.

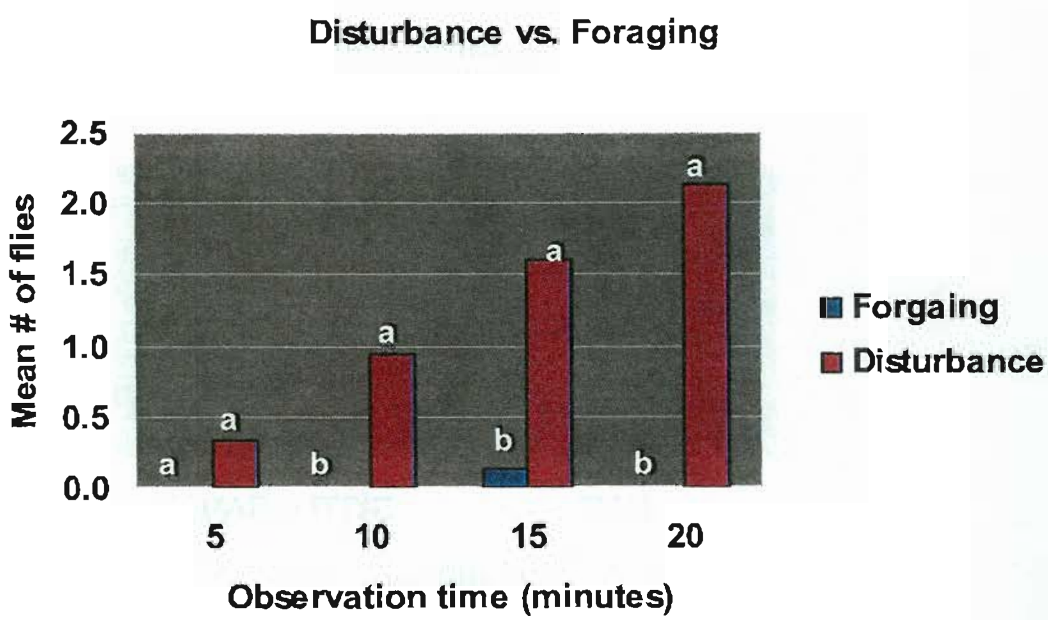


Figure 6.

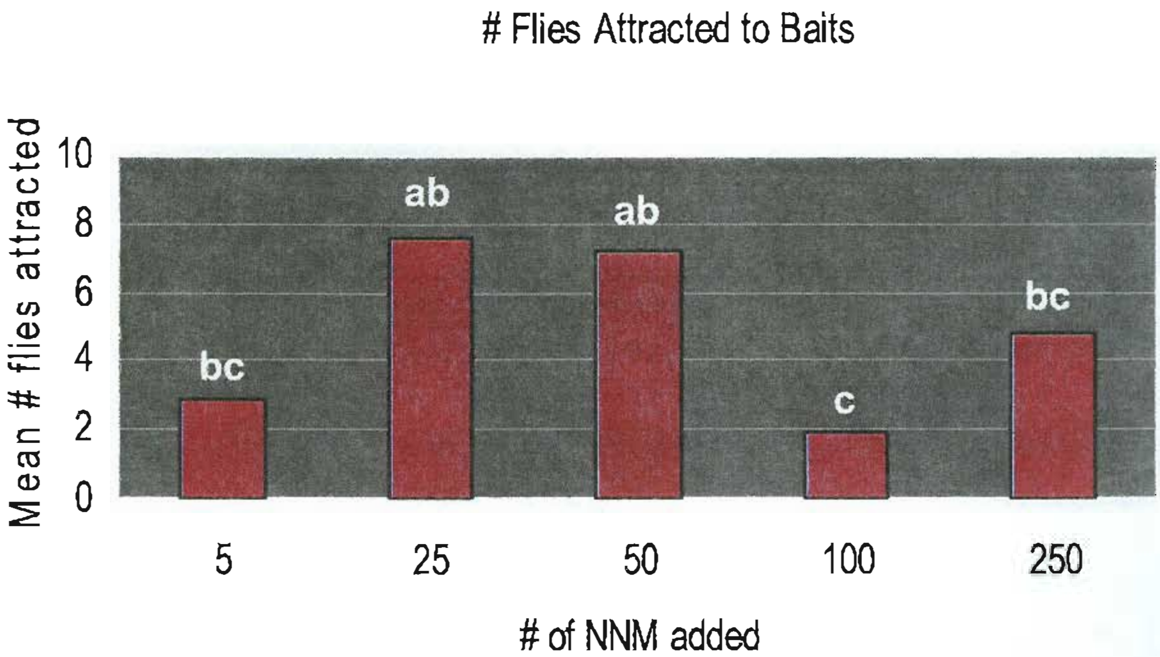


Figure 7.

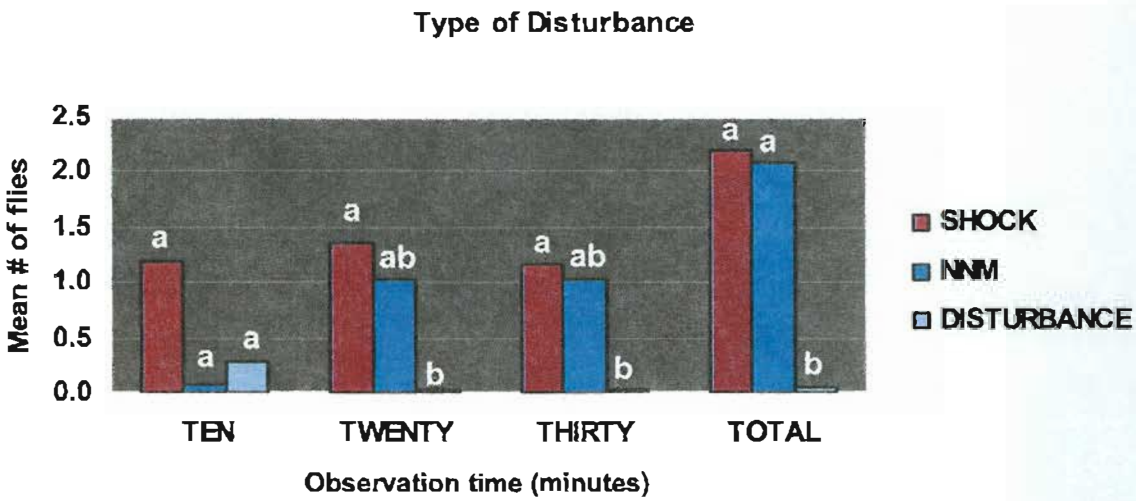


Figure 8.

Establishment and Spread of *Pseudacteon curvatus* in Tennessee

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Several releases of *Pseudacteon curvatus* and *P. tricuspis* were made in Tennessee from 1999 to 2004. A summary of these releases, including the estimated number of flies or parasitized workers released, is presented in the table below.

Species	Date	Cooperating Institutions[a]	Location	Estimated no. released[b]	Release method[c]
tricuspis	8/99	CMAVE, UT	Bradley Co.	2350	adults
tricuspis	9 -10/02	SIPS, DOACS, TSU	Franklin Co.	2730	adults
tricuspis	7/03	SIPS, DOACS,TSU	Franklin Co.	2170	adults
curvatus	4/00	CMAVE, UT	Bradley Co.	4500	workers
curvatus	5 - 6/00	CMAVE, UT, AP	Fayette Co.	4000	workers
curvatus	9 - 10/00	CMAVE, UT	Monroe Co.	4000	workers
curvatus	9 - 10/02	BCPRU, TSU	Franklin Co.	3400	workers
curvatus	9/03	BCPRU, TSU	Franklin Co.	2100	workers
curvatus	7/04	BCPRU, TSU, UT	Hamilton Co.	8200	workers

[a]CMAVE = USDA ARS Ctr. for Medical and Veterinary Entomol.; UT = Entomol. & Pl. Pathol. Dept., Univ. of Tennessee; SIPS = USDA APHIS PPQ CPHST ANPCL Soil Inhabiting Pests Section; DOACS = Florida Dept. of Agric. and Consumer Serv.; TSU = Inst. of Agric. & Environ. Res., Tennessee St. Univ.; AP = Ames Plantation; BCPRU = USDA ARS Biological Control Of Pests Research Unit. The Tennessee Dept. of Agric. cooperated with every release.

[b] Estimated number of adult flies released was about 40 to 60 parasitized workers per mound. Estimated number of parasitized workers released based on approximate number of ants exposed in the laboratory and measured parasitism rates for laboratory colonies.

[c]Adults: adult flies released at mounds; workers: IFA workers were exposed to ovipositing flies in the lab and then released back into their original mounds.

All of the release sites were pastures with the exception of the 2003 Franklin release of *P. curvatus* which was made at an ornamental nursery, and the 2004 release which was made at a cemetery. All releases of *P. curvatus* were of the Los Flores biotype except for the July 2004 release in Hamilton Co. where the Formosan biotype was released.

Sampling of imported fire ant (IFA) mounds at release sites in Bradley, Fayette and Monroe counties in 2000 and 2001 yielded no adult flies and the releases were considered failures. Collections of *P. curvatus* at the Franklin Co. pasture release site in July and August 2003 indicated the flies released the previous year had successfully overwintered and were established. Only one *P. tricuspis* adult has been collected: at the Franklin Co. release site in July 2003.

On July 13, 2004, while making the release of hybrid imported fire ant (HIFA) workers parasitized with *P. curvatus* Formosan biotype at a cemetery in southeastern Hamilton County near Chattanooga (the only release made at this site), J. Oliver and T. Rashid observed *Pseudacteon* adults hovering over disturbed IFA mounds. Flies were collected and identified as *P. curvatus*. The most likely source for this population was one or both of the releases made in 2000 in east Tennessee (Bradley and Monroe counties). This find prompted a multi-county sampling survey to determine the geographic range of *P. curvatus* in Tennessee.

IFA mounds were sampled at release sites and at approximate 8-km (5-mile) intervals along highways leading away from release sites. Sampling consisted of digging into a mound, crushing or electrocuting (with a modified cattle prod) workers to release alarm pheromone, and observing the disturbed area to detect and collect flies attracted to the disturbance. Collected flies were returned to the lab for identification. IFA workers were also collected from each mound sampled and sent to the lab of Dr. Robert Vander Meer, USDA-ARS-CMAVE, for identification using cuticular hydrocarbon analysis.

Mounds at 113 sites in 25 Tennessee counties were sampled for *P. curvatus*. Mounds located in northern counties of Alabama, Georgia and Mississippi were also sampled. *P. curvatus* was collected at 73 of the sites in 21 Tennessee counties, including all of the 2000 release sites. Sampling results indicate that the fly, although not detected in the year after its release, was apparently established at the release sites after the 2000 releases. The greatest distance a fly was collected from any release site was approximately 98 km (61 miles). Flies were collected at all six sites sampled in two Georgia counties; and in three of five sites sampled in four Mississippi counties. The flies collected in Georgia and Mississippi appear to be of the population originating from the 2000 releases in Tennessee. No flies were found at the four sites sampled in one Alabama county.

Results of the survey indicate *P. curvatus* has spread throughout most of the IFA-infested region in Tennessee. (Currently, all or a portion of 38 counties across the southern region of the state are under quarantine for IFAs.) Identification of IFAs collected from sampled mounds substantiated results of earlier collections to determine the distribution of IFA species in the state: *Solenopsis richteri* (the black imported fire ant or BIFA) predominates in the west and the HIFA is predominant in the east. Populations of *S. invicta* (the red imported fire ant or RIFA), which was collected during the 2004 survey from only one site (in east Tennessee), are rare.

Because BIFA is the natural host of the Los Flores biotype of *P. curvatus* in South America, and the biotype has shown host preference for BIFA and HIFA over RIFA in laboratory studies, the successful establishment and subsequent spread of the Los Flores biotype in Tennessee should not be surprising (even after the earliest releases were deemed failures). Sampling surveys will continue in summer 2005 to document further range expansion by *P. curvatus* in Tennessee.

Space to Place Phorid Fly Monitoring Systems

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GIS (geographic information systems) is a dynamic tool that can organize and compile relevant biotic and abiotic factors into an integrated decision support application. This approach can be of immense value in targeting areas for efficient and effective phorid fly releases into imported fire ant (IFA) populations. A GIS application is being developed as a web-based application for delivery to State collaborators as a decision and management system.

There are two components to the GIS application; 1) development of integrated phorid fly tracking/data systems, and 2) a predictive decision and management support program. Each component of the project is structured as a stand alone program with supporting data for the predictive model relying on data from the tracking component. The tracking component of the project has been initiated in the first two years of the multi-year project. The tracking program uses state of the art GIS and GPS technology to collect, display, and, organize information on phorid fly releases and IFA populations within states.

Spatial data are collected in two ways; 1) via web-based data entry forms maintained at NCSU's Center for Integrated Pest Management and 2) via handheld data collection units using a GPS (Global Positioning System) attached to a Pocket PC. Data are entered into GPS/Pocket PC units via customized application forms running in ARCPAD 6 (ESRI®). Application forms were designed using ARCPAD Application Builder 6 (ESRI®). Data are maintained using ARCGIS 8.3 (ESRI®) software in Gulfport, MS at the Soil Inhabiting Pests Section, http://www.cphst.org/sections/SIPS/.

Project Highlights:

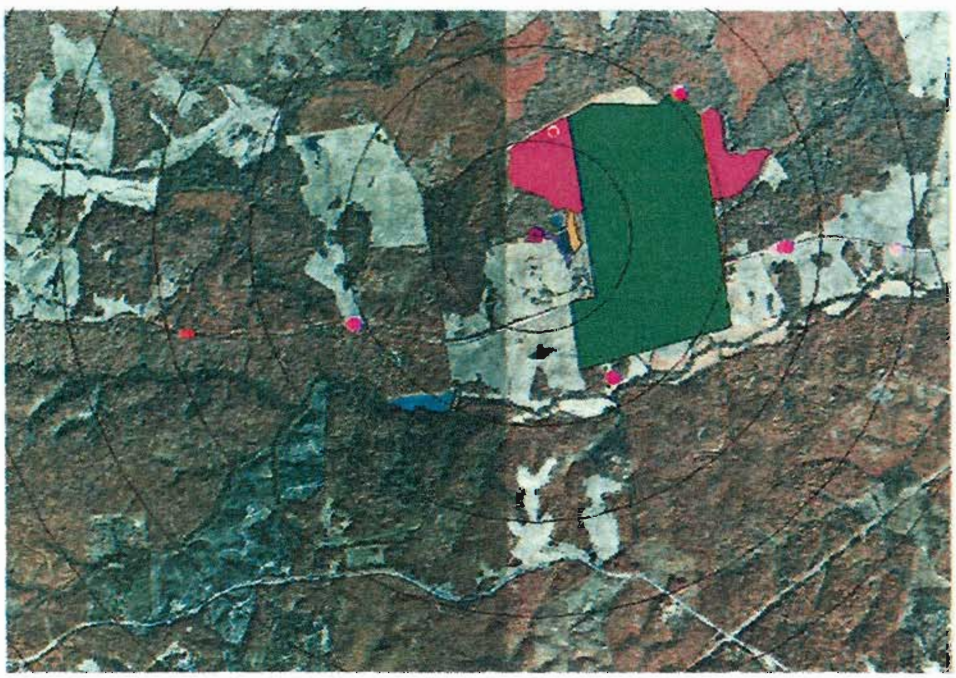
- Completed development and posting of web page describing project; http://www.cphst.org/projects/Phorid_monitoring
- Completed development and delivery of electronic data entry forms and PDA/GPS handheld units. Several units are available for use on request.
- Completed 2004 surveys in both Mississippi and Alabama using PDA/GPS hardware and software.
- Completed web-based data entry application – collaborative effort between CPHST and NCSU's Center for Integrated Pest Management: Website is currently available for collaborators at https://flydata.cphst.org/flyData/main.cfm Contact Ron Weeks at Ron.D.Weeks@aphis.usda.gov for a user login ID and password.

Pseudaction curvatus release and spread in South Carolina

T. Davis

Abstract:

Pseudacteon flies have been released at seven location in South Carolina since 1997. *Pseudacteon tricuspis* was release in Clemson, Pelzer, McEntire Air National Guard Base, Greeleyville (2002), Fairfield County (2002), and Horry County (2004). Overwintering and spread has been documented at the Clemson, McEntire, Greeleyville, and Fairfield sites. *Pseudacteon curvatus* has been release at two sites Fairfield County (2003) and Charleston County. The Charleston site was released in October of 2004 and overwintering has not been confirmed. The 2003 release in Fairfield County has overwintered at the site and spread more than a mile in each direction. Confirmed sites are demonstrated on the map below. The spread of *P. curvatus* at this site has been more rapid than any of the *P. tricuspis* sites in South Carolina. To our knowledge South Carolina is the northern most establishment of *P. tricuspis*. It is hypothesized that perhaps *P. curvatus* is more adapted to the cooler climates found in South Carolina.



Map of Fairfield Areawide Fire Ant Suppression site. Center of concentric circles is the epicenter of the P. curvatus release. Circles represent 2000 ft. furthest confirmed capture is approximately 8000ft from epicenter.

Thelohania Infection and Corresponding Decreases in Fire Ant Mound Densities

Charles L. Barr, Alejandro A. Calixto and Forrest Mitchell

As part of the USDA-ARS Area-wide Suppression of Fire Ants in Pastures project, two sites in Texas were established to monitor red imported fire ant (*Solenopsis invicta* Buren) suppression through the integrated use of broadcast baits and biological control organisms. Since the study began in 2002, these sites have been monitored semi-annually for various factors. As with many field trials, particularly very large, long-term ones such as this, the data collected can raise more questions than they answer. This article addresses two phenomena found at the Texas Area-wide sites: colonies that were not controlled by two bait applications, but were controlled by a third and; the relationship of increased *Thelohania* infection to decreased mound densities.

Materials and Methods

Each of the two field sites consists of a 300-acre area that was treated with a combination of aerially-applied broadcast baits: a 50:50 hopper blend of AmdroPro® (0.73% hydramethylnon) plus Extinguish™ (0.5% s-methoprene) applied at 1.5 lbs. per acre. Twenty, 1/8th-acre circular monitoring plots were established within each treatment area.. Thirty additional plots were established around each treated area to act as untreated controls.

Five Eagle Ranch in Burleson County, Texas serves as the "treated" site in which biological control organisms were present. Two species of phorid fly, *Pseudacteon tricuspis* and *P. curvatus* were released at the site with *P. tricuspis* establishing a vigorous infestation by the summer of 2003. The second organism of concern was *Thelohania solenopsae*, a microsporidian fire ant "disease" that has been shown to reduce colony vigor and reproductive capacity. *Thelohania* was naturally present across the Five Eagle test site at the beginning of the test, with 14 of the 50 plots testing strongly positive for the organism.

The second or "control" site was NK Cattle Company located in far north Brazos County, Texas along the Robertson County line. It is located approximately 25 miles from Five Eagle Ranch. No phorid flies were released near the site and none have appeared to date. At the beginning of the test, only four of the 50 plots tested weakly positive for *Thelohania*, so it was considered acceptable as a control site.

Plots were first treated with broadcast bait on May 30, 2002. Subsequent treatments were made on October 11, 2002, and April 15, 2004. The following evaluations were conducted in both spring and fall of each year: mound counts, foraging sampling with hot dog slices, pitfall trap sampling and collection of ants for *Thelohania* monitoring. *Thelohania* samples were shipped to Forrest Mitchell of the Texas Agricultural Experiment Station in Stephenville, TX for analysis by PCR and microscopy.

Data are only from untreated plots. One plot was omitted from the Five Eagle data due to repeated flooding, another was omitted from NK data due to accidental bait treatment. The *Thelohania* index was calculated by multiplying the number of infected plots by the overall infection rate. It was felt this was a more accurate representation of the state of infection. For instance, from fall 2003 to spring 2004 at NK, the number of infected plots dropped by half, the infection rate dropped by two-thirds.

Findings and Discussion

"The mounds that would not die." After the first bait treatment in May 2002, we noticed an area along the northeastern edge of the NK Cattle Company site where colonies seemed to still be present in large numbers. In the spring of 2003, after the second bait application, large mounds with active colonies were still readily visible in a strip approximately 100 yards wide and running the length test site, about one mile. Colonies in the rest of the treated area, sometimes only a few yards away, were controlled quickly and completely.

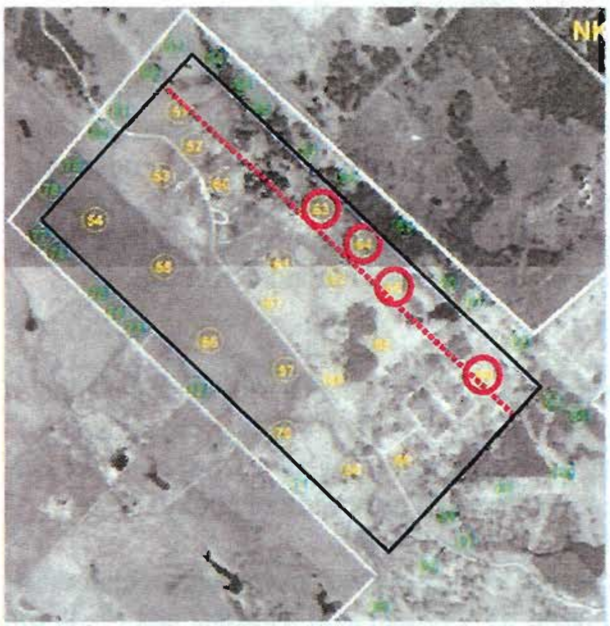


Figure 1. "Persistent" fire ant colonies at NK Cattle Company.

Fortunately, four monitoring plots were located within the strip of poor control (see Fig. 1). A review of the data confirmed that mound density in these four plots did not just fail to decrease after two treatments, it actually increased 28% compared to pre-count levels. (See Fig. 2). The third treatment in April, 2003 finally killed these colonies.

The authors are at a loss to explain the failure of two bait treatments to control fire ant colonies in this particular strip of land, then obtaining good control with a third treatment. The products used were the same. Because of a broken GPS antenna, we were forced to use balloons to mark the swaths during the second application. All three personnel reported bait falling on them while in the area in question. The strip was treated first in the morning during the first application, but last during the subsequent two. Weather was not a factor given the good control across the rest of the treated area.

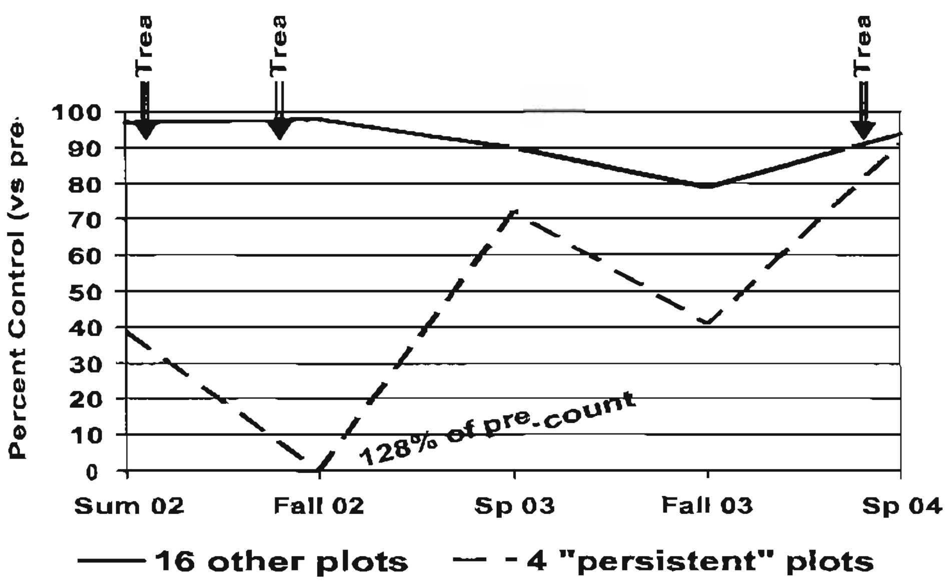


Figure 2. Percent colony control with broadcast bait at NK Cattle Company

"The rise and fall of a natural Thelohania infection." For reasons that are still unknown, the *Thelohania* infection rate increased dramatically at both sites over the course of the summer of 2002. Most dramatic was the increase at our "control" site, NK Cattle Company, where four of 50 infected plots increased to 29. Though this natural phenomenon confounded any attempt to compare the effects of the presence of *Thelohania* between the two test sites, it did provide the opportunity to study the effects of a sudden *Thelohania* infection in a previously uninfected (or lightly infected) area - an opportunity that we could not possibly have designed a test for.

As shown in Figures 3 and 4, *Thelohania* infection rates increased steadily at both sites through the fall of 2003. Accompanying the increase in infection rate was a steady decrease in mound density at both sites. However, the infection index dropped markedly at both sites in the spring of 2004, with little appreciable change in mound density.

As with the persistent mounds, these results raise more questions than they answer. What caused the dramatic spread of *Thelohania* over the summer of 2002? Similarly, what caused its precipitous decline over the winter of 2003-4? Could weather be a factor since the test was established at the end of a drought and 2004 was the third wettest year on record? Is this simply a natural cycle that we have not followed long enough to delineate?

Regardless of anything else garnered from the Area-wide sites, they have given us the opportunity to study fire ant infestations on a large scale over the course of several years. We hope to learn more as the demonstration progresses.

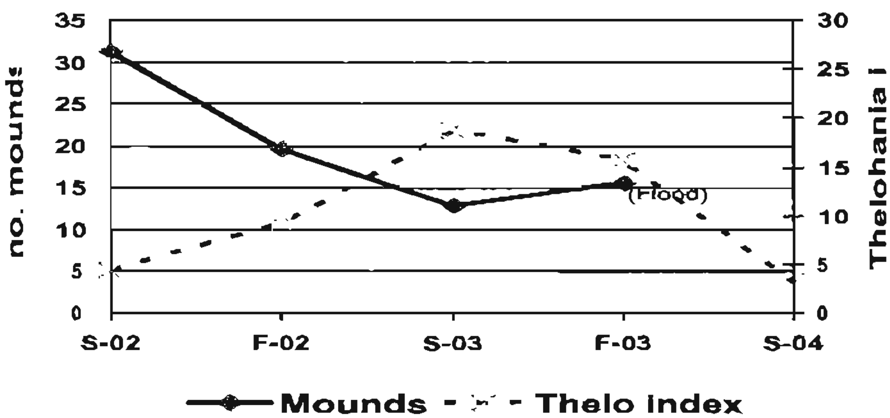


Figure 3. Mound counts vs *Thelohania* index at Five Eagle Ranch

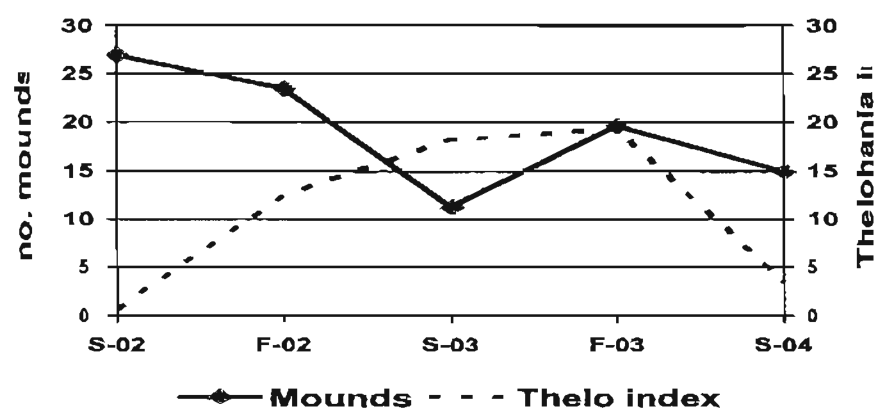


Figure 4. Mound counts vs *Thelohania* index at NK Cattle Company.

Use of baits for evaluation of fire ant populations in the USDA areawide project

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In sites used in the USDA-ARS Areawide Fire Ant Suppression project, fire ant populations are monitored using baits (ant activity monitoring) or mound counts (mounds rated using the USDA Population Index [PI] rating). For ant activity monitoring, 10 slices of beef wieners are placed about 8-paces apart in a circle around the sampling plot centers. After 30-60 minutes, baits are observed and rated from 0-4 depending on number of fire ants present at the bait. These observations allow the calculation of % of baits where IFA are present. All mounds present in the sampling plots are counted and given a rating varying from 1-10 depending on the number of ants in the nest and the absence (rating = 1-5) or presence of brood (rating = 6-10).

The treatment threshold used in this program is 20 mounds with PI>7 per acre, but no threshold has been established using the ant activity monitoring baits. Therefore, we were interested whether the ant activity as measured in the beef wiener baits corresponded to the level of ant population as measured by the mound counts and PI rating. This is important because the ant activity monitoring is: easier to use, less labor- and time-intensive, more efficient in detecting low populations, and serves as an indicator of adequate time for chemical bait applications because it determines if foraging is occurring.

Data from all 5 cooperating states show that there is a strong correlation between the ant activity as measured by the baits and the ant population as measured by number of mounds or the PI. Although the correlation is stronger when using the rating for the number of ants present at the baits, the percentage of baits occupied by fire ants also provide reliable information, with lesser requirement in effort and technical knowledge by the operator. The ant activity bait responds rapidly to an increase in ant population, and baits can be quickly overwhelmed by fire ants if populations are above levels usually considered damaging and in need of control. The pre-established threshold (20 mounds/acre) corresponds to 30-45% of the baits occupied by fire ants.

In a simulation in the laboratory, the ant activity bait data were used as the decision tool in determining the need for fire ant control treatment in the different demonstration sites for the areawide project over the past 3-4 years. The decisions to apply formicides were similar in >80% of the times whether the mound counts-PI or the ant activity bait (% of bait occupied by fire ants) were used. Variation of the data negates any differences in the decisions when the different methods were used.

In conclusion, ant activity monitoring baits (slices of beef wieners) are good indicators of fire ant populations in pastures. Compared to mound counts-PI, ant activity baits tend to underestimate the ant population in cooler months and overestimate the ant population in warmer months. However, because they are easier and less expensive to use, the ant activity monitoring baits can be recommended as a simple, user-friendly method for evaluation of fire ant populations, and as a decision tool in fire ant control programs.

An image tracking technique for quantifying motion of black imported fire ants on a planar surface

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Abstract
An image tracking technique that is similar to the particle image velocimetry (PIV) is described in this paper to quantify motion of ants on a planar surface. This measurement technique is based on a digital imaging system that successively acquires image frames with a higher frame rate and resolution than a standard video system. The constant background noise is extracted by averaging hundreds of frames and removed from each frame. The individual ant images are identified and paired between frames according to the size, brightness, shape, and orientation angle of the ant image. The speed of an individual ant is determined with the displacement of its images and the time interval between frames. This measurement technique is applied at the University of Mississippi's National Center for Physical Acoustics (NCPA) for investigating reactions of black imported fire ants (*Solenopsis richteri Forel*) to substrate vibration of a selected frequency. Experimental results indicate that the fire ants are sensitive to the vibration excitation, i.e. they move faster after being excited, however, the numbers of active ants are not obviously increased even after a strong excitation.

1. Introduction

A vibration-based technique is under development at the University of Mississippi's National Center for Physical Acoustics (NCPA) for inducing motion of black imported fire ants (BIFAs) in a tub. The tub is normally situated inside of large "attack boxes" used by research entomologists at the Biological Control of Pests Research Unit (BCPRU) in Stoneville, Mississippi, to provide an arena whereby worker ants are exposed to attacks by parasitoid phorid flies (*Pseudacteon curvatus* Borgmeier) in a dedicated effort to increase artificial mass production of phorid fly pupae inside the bodies of fire ant workers (Porter 2000, Vogt 2002). In order to quantify the distribution and motion of the fire ants in the tub, a digital imaging system is used to record successive image frames of fire ants on the tub bottom surface. The digital ant image recordings are processed in a similar way to those for processing the particle image velocimetry (PIV) recordings of low-image-density (LID) mode.

PIV is an optical measurement technique for fluid flows seeded with tiny tracer particles (Merzkirch 1990, Cenedese and Paglialunga 1990, Adrian 1991, Willert and Gharib 1991, Grant 1997). In a standard PIV system a laser light sheet is used to illuminate a plane in the measured flow field. Image pairs are usually taken with a digital camera to record displacements of tracer particles in the plane during a selected time interval. Many different algorithms can be used to determine the particle image displacements in a digitized PIV recording pair so that the velocities of the flow tracers can be calculated. The velocity of a tracer particle reflects the fluid flow velocity around the tracer particle. The PIV technique can be modified and applied to the ant motion measurement. To fit PIV to the ant tests, the following factors should be considered: (1) Since the ant motion can be limited in a planar surface, the laser light sheet can be omitted. However, the shadow of the ants on the surface should be avoided because the size of an ant is much larger than a tracer particle. (2) To capture moving objects in a natural illumination situation, the digital camera should have a high sensitivity to the light so that a short enough shutter time can be used. (3) Without a planar illumination, the noise in the background that includes reflection, shadow and unexpected objects is recorded together with the images of ants. Therefore, image-processing techniques are required to extract ant images from the disturbed raw recordings. (4) Because the distribution density of ant images is much lower than that of the particle images in usual PIV recordings, a particle image tracking (PTV) algorithm should be used to determine the ant image displacements, thus, suitable methods are required to identify and track individual ant images. In the present work the first two factors, i.e. (1) and (2), were taken into account at the hardware selection and system setup, whereas the last two factors, i.e. (3) and (4), were considered by developing appropriate image processing methods and evaluation algorithms.

Many different algorithms have already been developed to track particle images in LID PIV recordings, e.g. Chang et al. (1985), Okamoto et al (1995), Baek and Lee (1996), Cowen and Monismith (1997). In most PTV algorithms, the centers of particle images are identified to determine the particle image displacements, and the particle images are paired according to their relative position to spatially or temporally neighbored particle images. Because the images of tracer particles are usually very small, very few PTV algorithms use the information of particle size, brightness and shape. The authors constructed an image tracking technique for fire ants based on methods and algorithms presented by Gui et al. (1996, 1997) and Gui (1998). Gui et al. (1997) described a method for extracting and removing background noise from a group of LID PIV recordings. That method exactly fits the present work for processing the ant images. A recursive digital filter (Gui, 1998) demonstrates good performance in identifying both solid particle images and fire ant images. Gui et al (1996) developed a two-frame algorithm for tracking big solid particles in a multi-phase tank by using information of particle size, brightness and shape. Since the ant images have similar characters as those of the big solid particles, this two-frame tracking algorithm is modified and applied in the ant tests. The authors implemented the image processing methods and algorithms in EDPIV, i.e. a software package for digital PIV recording evaluation, so that the image tracking technique can easily be used.

Even though the motion of an individual ant can be tracked from time to time by using the described image tracking technique, the present work was focused on the overall reaction of a fire ant group to substrate vibration of a selected frequency. Tests were performed with a group of fire ants of known numbers, so that the ratio between the active moving ants and those staying calm in clusters can be determined. A probability density function (PDF) was determined with instantaneous ant speeds of all the active moving ants in a time period. The selection of exciting frequency was based on a previous tub vibration mode analysis conducted by Khoo et al (2003), so that the ants in the tub can effectively be excited by the substrate vibration. In order that the ant reaction is natural, experiments were conducted in an isolated room to reduced unexpected disturbances, and a long enough rest time were set between excitations.

In this paper we shall at first introduce some details of the experiment. Then, the image processing method and evaluation algorithms will be described. Finally, typical test results will be presented and discussed.

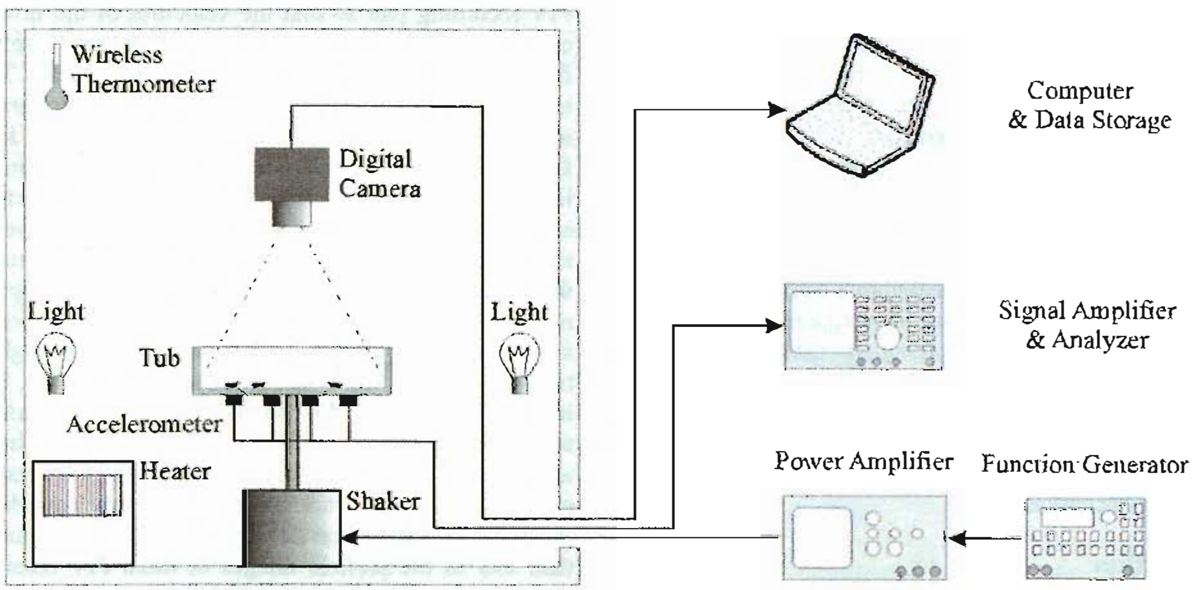

Fig. 1: Schematic illustration of experiment setup

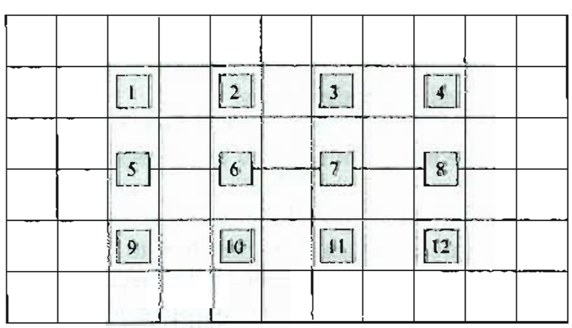

Fig. 2: Distribution of accelerometers on the tub bottom (distance between grid lines: 31.75 mm)

2. Experiment setup

As shown in Fig.1, a small isolated room measuring about $2\times2\times2$ m^3 was used as the experimental chamber. A small space heater was used to regulate the ambient temperature to 26-28 °C, which was monitored by using a remote wireless thermometer from outside of the chamber. As a play stage for fire ants, a plastic tub measuring $350\times190\times120$ mm^3 was placed in an aluminum support frame, confining the perimeter of the base of the tub but allowing for general deformation of the bottom surface of the tub. The 120-mm sides of the tub were coated with Fluon® AD1 to prevent the ants from escaping. The 350×190 mm^2 inner bottom of the tub was painted with grid lines of distance of 31.75 mm (1 ¼ inches) for vibration mode analysis and image calibration. Since the vibratory sensory organs of fire ants appear to be most sensitive to the acceleration of the substrate vibration (Mwangi et al, 2003), 12 units of accelerometers (Measurement Specialties ACH-01) were attached to the outer bottom surface of the tub to measure the vibration response. The distribution of the accelerometers on the tub bottom is shown in Fig. 2, overlapping with grid lines painted on the inner bottom of the tub. The tub support frame was connected to the center insert of a shaker (Brüel and Kjaer type 4808 vibration exciter) by a steel rod. The shaker was placed on a vibration absorbent pad in order to minimize the effects of ambient seismic energy. Four small halogen lamps served as light sources for imaging purposes, and they were so placed that no light rays directly reach the ants from the sources to prevent shadows on the background surface. A 10-bit CMOS digital camera with 17-μm pixels (Photron ultima APX) was attached to a tripod and fixed to a working distance of 600-mm to capture images of the fire ants before and after excitation. The CMOS camera has a memory big enough to save more than 1,200 frames in each of 10 separate zones, so that image groups of multiple tests can be acquired without stop for data downloading. Using XENON 0.95/25 C-mount lens, the camera acquired image frames of 1024×512 pixels at a frame rate of 60 fps, which covered an area of 418×209 mm^2 in the objective plane with an imaging scale of 2.45 pixel/mm. The exposure time is set to 1/500 second so that sharp images of moving ants were obtained.

Fig. 3: Sample image taken at the bottom of the test tub. (An ant cluster on the lower left corner, a food source at 8th grid line from left and 4th grid line from top, a white arrow indicating ant used in Fig. 9)

Other equipment components were placed outside of the room to minimize human interferences with fire ant behavior. The shaker was driven by the output of a power amplifier (Brüel and Kjaer type 2719), which was controlled in voltage mode with a function generator (Hewlett Packard model 3314A). The vibration signals acquired at the tub bottom were amplified using low-noise amplifiers (Measurement Specialties IB-ACH-01) and processed using a dynamic signal analyzer (Stanford Research Systems SRS model 785). Calibration of the analyzer was performed accordingly. A computer was used to control the image acquisition and to download images after a test case was completed. Image processing and evaluation were completed offline.

BIFA colonies were collected on the ground of the NCPA by excavating conical mounds and placing the ants and soil in talc-powder-coated five gallon buckets. Then they were extracted from the buckets after drip irrigation supersaturated the soil, displacing all of the ant castes to the surface of the water. Ants were collected using a strainer and placed in plastic tubs measuring 52.1 × 39.4 × 12.7 cm^3. The sides of each tub were also coated with Fluon® AD1 to prevent the ants from escaping. The ant tubs were then placed inside of a Percival Scientific incubator maintained at 40% relative humidity and temperature of 27 °C. Colonies were provided with *Heliothis virescens* pupae as a food source and cotton-plugged 20 × 150 mm test tubes containing water and/or 20% water-sugar solution for moisture purposes. Worker ants used in this investigation were then collected from a single colony by vacuum extraction, counted, and deposited onto the surface of the tub inside the experimental chamber room. Acclimation to the new environment was allowed for 24 hours prior to commencement of experimentation, with drip irrigation providing a source of moisture (water-filled test tubes were avoided to prevent mass-loading of the tub surface). Lighting inside the chamber room was regulated by turning the lights on at ~ 8:00 AM every morning and then turning them off at ~ 5:00 PM to simulate the daylight cycle. 200 workers of fire ants were put into the tub for the tests.

Five tests were planned with the same ant group on five consecutive days. According to Khoo et al (2003), the exciting frequency was selected to be 466 Hz to effectively stimulate the fire ants distributed on the tub bottom. Different vibration intensities were applied on different days. On each day, a test started around 3:00 PM, and 10 groups of fire ant images were recorded. The first ant image recording group was taken one minute before the excitation as a reference. The second ant image recording group was acquired a few seconds after the ants were excited. The remaining 8 ant image recording groups were obtained at different time delays to observe the development of the ant motion within a one-hour period. Each image group has 1228 frames that were acquired at a rate of 60 fps in about 20 seconds. A sample image is shown in Fig.3. In this sample image, most of the ants cluster on the lower left corner of the image frame; some of the ants randomly distribute on the tub bottom; and a food source of two *Heliothis virescens* pupae is placed on the mid-high right.

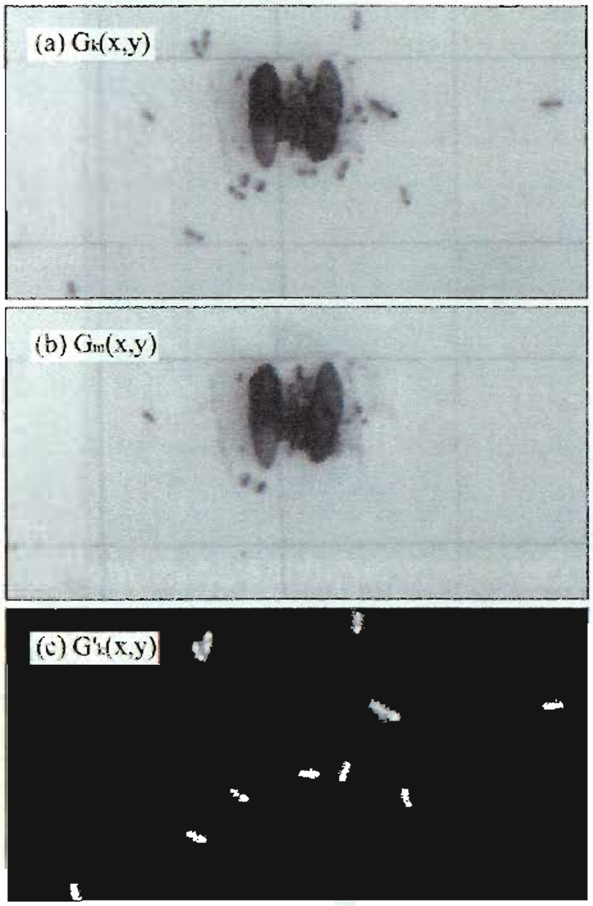

Fig. 4: Sample images (256×128 pixels). (a) Raw image, (b) Averaged, (c) Background-removed.

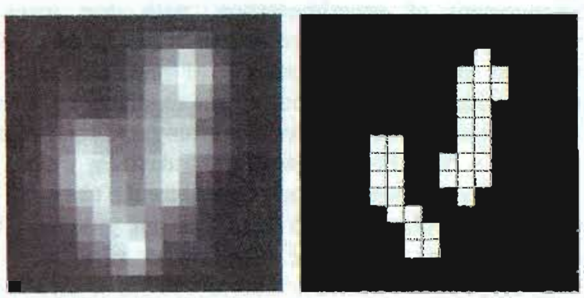

Fig. 5: Sample image including 2 ants (16×16 pixels). (left) grayscale image, (right) binary image.

3. Image processing and evaluation

3.1. Removing background disturbances

As shown in Fig. 3, the bottom of the tub is not uniformly illuminated, and the painted grid lines and unexpected objects like the food source, dust and dead ants are imaged together with the investigated active moving ants. The ant image recordings must be cleaned before being evaluated to ensure reliable and accurate results. The method described by Gui and Merzkirch (1997) can be used to effectively extract the image of the constant background disturbances and to remove it from each ant image recording. Fig. 4a shows a part of a raw ant image recording of size of 256×128 pixels. In the raw image sample the grid lines, food resource, dead ants, and dust pieces are included. In the averaged image, i.e. Fig. 4b, some of the ant images disappear because they are images of active moving ants whose contribution to the averaged gray value is divided by 1228. When the background disturbances are removed by using equation (2), only images of active moving ants stay in the processed image frame, see Fig. 4c. Note that the ant images in Fig. 4c are inverted.

Table 1: Example of ant image identification results

n	X	Y	S	B	Θ	θ
1	30.33	4.33	15	170.1	1.86	-1.27
2	84.29	28.11	17	171.1	2.25	-0.37
3	176.43	45.68	16	166.7	2.39	-1.25
4	103.00	46.50	16	167.7	2.55	-0.62
5	149.56	56.81	16	172.9	2.95	1.23
6	133.53	55.66	15	163.9	2.60	0.05
7	167.62	83.31	39	169.4	2.69	-0.47
8	241.65	85.94	17	172.4	2.15	0.15
9	85.21	110.00	14	190.6	2.26	-1.20
10	89.50	114.00	20	176.4	2.00	1.41
11	155.50	122.25	16	171.5	2.63	1.55

3.2. Image identification

The tracking of individual ants between image frames is based on ant image identification, i.e. determination of the position and characters of each ant image. In order to complete image identification, the processed digital image recording as shown in Fig. 4c is binarized according to a selected gray value threshold, so that the boundary of the ant image is fixed. The binary effect of the ant images is shown in Fig.5. In the presented case, the binarized ant images consist of teens to tens of square bright units (pixels of gray value 1) in the dark background (pixels of gray value 0). When the binarized ant images are identified with the method described by Gui (1998), the total number of ant images (N), the number of pixels (i.e. size) of each ant image (S), and the position of each pixel for certain ant image are determined, that enables a further determination of the position (X,Y) and brightness (B) of the ant images. In addition, the roundness (Θ) and orientation angle (θ) can also be determined to represent the shape characters of an ant image. As an example, the ant image identification results for image sample in Fig. 4c are given in Table 1. The size of the eleven (i.e. N=11) identified ant images vary from 14 to 39 pixels. In this case, the maximum round up error for determining the ant image center position can be estimated as 0.5/14=0.036 pixels. If the ant image displacement is more than 1.5 pixels, the relative error will be smaller than 3%, that is acceptable in most engineering tests. Therefore, it is reasonable for using the identified center position to determine the ant image displacement, and consequently, to determine the ant moving speed.

3.3. Image tracking algorithm

Assume there are M_o and N_o numbers of ant images in the first and second frame of an ant image recording pair, and the image identification results for the two frames are represented as $\{X_{1,m}, Y_{1,m}, S_{1,m}, B_{1,m}, \Theta_{1,m}, \theta_{1,m}$ for $m=1,2,3\cdots,M_o\}$ and $\{X_{2,n}, Y_{2,n}, S_{2,n}, B_{2,n}, \Theta_{2,n}, \theta_{2,n}$ for $n=1,2,3\cdots,N_o\}$, respectively. A tracking function is defined as below to quantify the likeness of two ant images.

$$D(m,n)=c_S\left[\frac{S_{2,n}-S_{1,m}}{S_{1,m}}\right]^2+c_B\left[\frac{B_{2,n}-B_{1,m}}{B_{1,m}}\right]^2+ c_\Theta\left[\frac{\Theta_{2,n}-\Theta_{1,m}}{\Theta_{1,m}}\right]^2+c_\theta\left[\frac{\theta_{2,n}-\theta_{1,m}}{0.5\pi}\right]^2 \quad (1)$$

Wherein c_S, c_B, c_Θ and c_θ are weighting coefficients. Tracking function $D(m, n)$ describes a sum square difference of weighted variation rates of image size, brightness, shape, and orientation angle between image m in the first frame and image n in the second frame. The orientation angle θ may not be used in usual PIV for tracking spherical particles, e.g. Gui et al (1996), however, it may dominate the tracking because many ants have similar size and shape, and their images have the same brightness level. The criteria to judge that image m' in the first frame and image n' in the second frame are images of the same ant is given as

$$D(m',n')=\min\left\{\begin{array}{c} D(m',j)\ \ for\ j=1,2,3\cdots,N_o\ and \\ (X_{2,j}-X_{1,m'})^2+(Y_{2,j}-Y_{1,m'})^2\le R_s^2 \end{array}\right\} \quad (2)$$

wherein search radius R_s is used to limit the search for the image partner n' in the neighbored area of the tracked image m', so that some false tracking can be avoided and the computation time can be reduced. The final results of the two-frame image tracking are position (X,Y) and displacement $(\Delta X, \Delta Y)$ of the tracked image pair, i.e.

$$\begin{cases} X(m',n')=(X_{2,n'}+X_{1,m'})/2 \\ Y(m',n')=(Y_{2,n'}+Y_{1,m'})/2 \\ \Delta X(m',n')=X_{2,n'}-X_{1,m'} \\ \Delta Y(m',n')=Y_{2,n'}-Y_{1,m'} \end{cases} \quad (3)$$

The speed of the ant is then determined by

$$\begin{cases} V_x(m',n')=\dfrac{\Delta X(m',n')}{\Delta t} \\ V_y(m',n')=\dfrac{\Delta Y(m',n')}{\Delta t} \end{cases}, \quad (4)$$

wherein Δt is the time interval between the two frames in the ant image recording pair.

4. Experiment results

According to the original plan the tests were conducted on five days. On the first two days the fire ants performed very well, however, the ants began to die on the third day. Since insufficient numbers of ants were captured on the last three days, the results deduced from data taken on those three days are not reliable. Fortunately, the data acquired on the first two days provided sufficient information for our test purpose. In the following we refer to the test case on the first day as case #1 and the test case on the second day as case #2. The excitation applied in case #1 resulted in the maximal acceleration amplitude of 0.72 m/s^2 near the tub center at sensor position 7 in Fig. 2. The exciting signal applied in case #2 resulted in the maximal acceleration amplitude of 82.13 m/s^2 at the same position. Case #1 was considered to be a case of weak excitation, whereas case #2 was of very strong excitation.

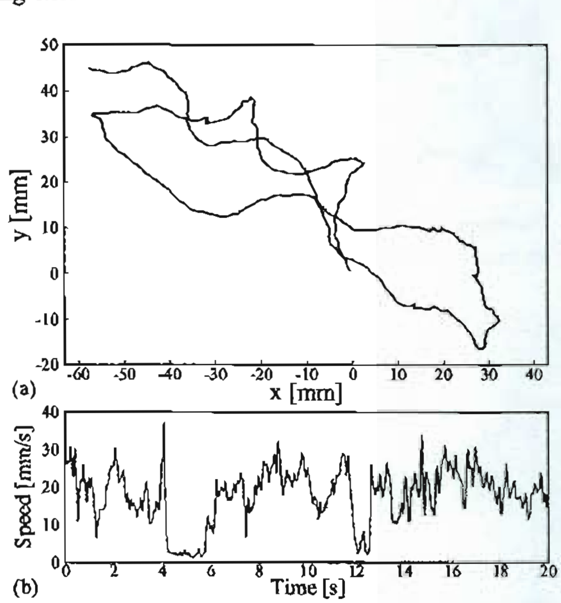

Fig. 9: Trail (a) and speed (b) of a selected ant in 20 seconds.

With the described image tracking technique the trail and speed of each active moving ant were determined in a 20-second data acquisition period. As an example, Fig. 6 shows the trail and speed time history of the ant indicated with a white arrow in Fig. 3, which belongs to the ant image recording group in case #2 for a few seconds after the excitation. Fig. 6a shows that the trail of the ant starts at position (0,0), goes along a "∞" shape in a region of 90×60 mm^2 in the 20-second period. In Fig. 6b the ant speed varies between 0 and 40 mm/s with a very complex distribution on time. The tracking results for a single ant enable study of the individual ant behavior. However, in the present work we concentrated on the overall behavior of a group of 200 ants. Fig. 7 displays the overlapped positions (7a) and speed vectors (7b) of identified 62 active moving ants in 1228 frames, which are evaluation results of the second ant image recording group for case #2. In Fig. 7a the trail of an individual ant can hardly be identified, however, the overlapped ant positions present a distribution of ant appearance probability in the observed area. Fig. 7b shows the corresponding ant moving speed vector at each ant position. Since so many vectors are overlapped, the speed of an individual ant cannot be seen clearly.

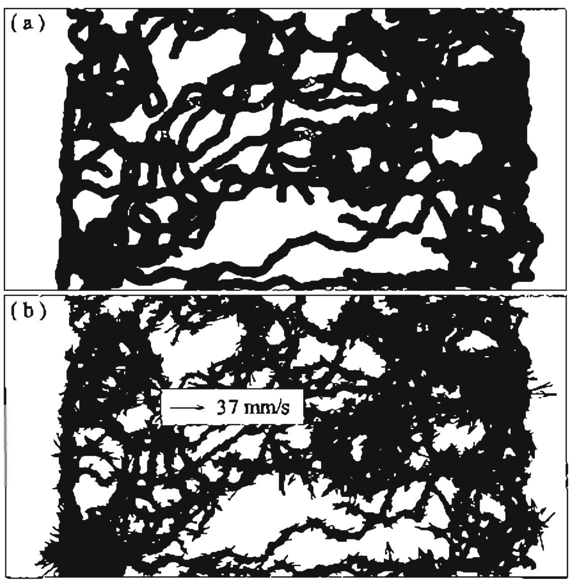

Fig. 7: Overlapped ant positions (a) and speed vectors (b) in case #2, 2nd group (view area: 418×209 mm^2)

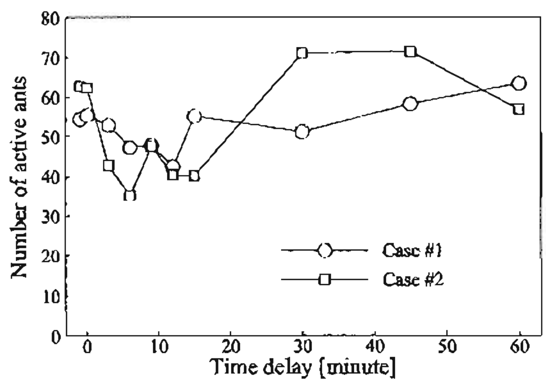

Fig. 8: Number of active ants in two test cases during 1 hour test period (total ant number: 200)

The evaluation results are further processed, so that more useful information can be extracted from the available raw data. At first, the number of the active ants is averaged in each recording group. The averaged active ant numbers for all the ant image recording groups in both cases are given in Fig. 8 in the one-hour test period. For each case the first symbol on the left represents the active ant number before excitation, and the second symbol, i.e. at "time delay"=0, represents data obtained a few seconds after the excitation. The followed eight symbols represent data obtained in the time period of restoration. As shown in Fig. 8, test results of both cases indicate that the number of active moving ants reduces after being excited by surface vibration of the tub, and it reaches to the minimum at around 10 minute of the time delay. After 30 minutes, the number of the active moving ants is back to normal, i.e. the number before excitation. After 40 minute the active ant number increases to a little higher than that of before excitation.

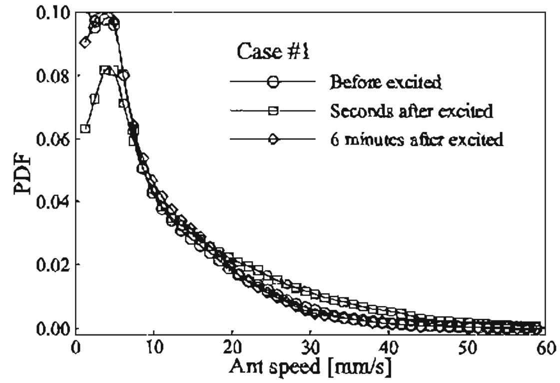

Fig. 9: PDFs of ant speed for Case #1

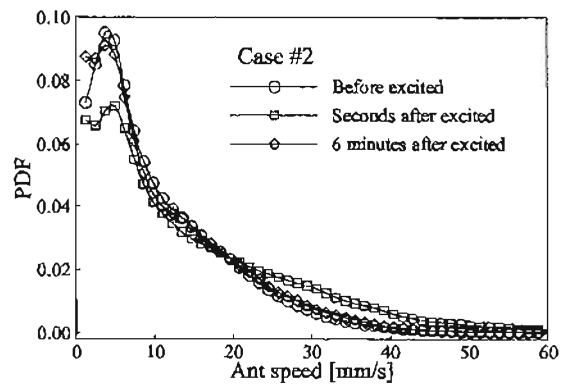

Fig. 10: PDFs of ant speed for Case #2

A probability density function (PDF) of ant moving speed is determined for each ant image recording group with about 36000 speed values as shown in Fig. 7b. The probability density functions (PDFs) of ant speeds determined before excitation, a few seconds after excitation, and 6 minutes after excitation are given in Fig. 9 and 10 for the two test cases, respectively. The PDFs of the two test cases are similar before the ants being excited, and only a little difference can be observed between 10 and 30 mm/s when they are plotted together. The undisturbed PDFs have a maximum near to 5 mm/s, and they reduce exponentially with increasing ant speed. It can be seen in Fig. 9 and 10 that the PDF of ant speed is obviously changed in a few seconds after the excitation, but it returns to the undisturbed distribution in about 6 minutes. The excitation of the tub surface vibration reduces the probability density of the low ant speed but increases that of the high ant speed. Obviously, the stronger excitation (i.e. in case #2) makes the moving ants a little more active, i.e. at a higher speed level.

5. Summary and conclusions

In this paper an image tracking technique is introduced for investigating moving ants on a planer surface. This technique includes a digital imaging system, a digital image processing method, an image identification tool, and an image-tracking algorithm. The digital imaging system requires a Mega-pixel imaging sensor, an adjustable frame rate to catch different moving ant speed, and a capability to consecutively acquire thousands of image frames. The image processing method was once applied in LID PIV and works perfectly for removing the constant background disturbances in digital ant image recordings. The image identification tool effectively detected the imaging region of each ant, so that its position, size, mean brightness, and shape information can be determined. The ant image tracking algorithm is similar to that used in a previous work for big solid particles in a water tank, but more effective, because the image orientation angle is added to the tracking parameters. Since the ants do not move vertically to the objective plane as particles in PIV tests, tracking an individual ant in its time history becomes possible.

The described image tracking technique was applied in the National Center for Physical Acoustics (NCPA) at the University of Mississippi for investigating the reaction of black imported fire ants (*Solenopsis richteri Forel*) to excitation with substrate vibration of select frequency. Tests were conducted with a group of 200 fire ant workers in a plastic tub. The experiment shows that, after acclimation to the environment for 24 hours, around three quarters of the ants keep calm in clusters, whereas about one quarter of the ants move actively. The speed of the active moving fire ants increases directly after the excitation, and return back to the normal level in about 6 minutes. The ant speed level is a little higher after a strong excitation than after a weak excitation. Experiment results indicate that the number of the active moving ants does not increase as expected after the excitation with substrate vibration, in the contrary, it decreases within 10 to 20 minutes after the excitation.

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Correlation of Colony Social Behavior and Infection with *Thelohania solenopsae* (Phylum Microspora) in Red Imported Fire Ants.

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The red imported fire ant, *Solenopsis invicta*, has become one of the most problematic introduced insect pests in the southern United States. These invasive ants are responsible for urban, agricultural crop and livestock production losses as well as medical costs. These ants are expanding in their range, and environmentally friendly control approaches are being sought in addition to widespread use of pesticides. The microsporidian parasite, *Thelohania solenopsae*, was first described in South American ants in the 1970's, and was first identified in the USA in 1998. This ant pathogen has been shown to have multiple negative impacts that devitalize infected ant colonies. Therefore, the parasite is a good candidate to explore as a potential biological control agent.

Fire ant colonies display varied social behaviors which are linked to allelic variation in the *Gp*-9 gene. Monogyne colonies have a single reproducing queen and large aggressive workers, while polygyne colonies have multiple reproducing queens and less aggressive workers. The goal of this project was to determine the colony social status for archived fire ant samples from all of the fire ant-infested counties in Texas, and to correlate these data with parasite infection status. Thusfar, 99 samples from 60 counties have been analyzed for social status. DNA was extracted from individual archived ants using an ammonium acetate-isopropanol extraction method. Two PCR amplifications were performed for each sample employing primer sets 26BS/16BAS and 24bS/25bAS using a modification of a previously described method to characterize each sample as monogyne or polygyne. Those genotypes were geographically recorded and correlated with parasite infection status.

Of the 99 samples, 37 colonies were identified as monogyne and 62 were identified as polygyne. Both colony types were dispersed throughout sampled Texas counties. Five of 37 monogyne colonies (13.5%) were positive for *T. solenopsae* while 30 of 62 polygyne colonies (48.4%) were parasite positive. This is the first extensive survey of naturally infected *T. solenopsae* colonies that correlates social status with parasite infection. Our molecular data showing a high parasite prevalence in polygyne colonies support previous field observations that suggested that polygyne colonies were more frequently infected with *T. solenopsae* than monogyne ant colonies. Thusfar, the geographic distribution of polygyne/monogyne colony types does not appear to correlate with the geographic distribution of the parasite. Knowledge of host-parasite relationships is important in evaluating the potential of *T. solenopsae* as a biological control agent against the red imported fire ant.

Defensiveness of the fire ant, *Solenopsis invicta*, increases during colony rafting

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Colonies of the fire ant, *Solenopsis invicta*, can survive flood conditions by forming a mat, or raft, of tightly grouped ants that floats on the water's surface until the flood recedes or higher ground is found. Forced from the protection of their nests and left without retreat, rafting colonies are both exposed and cornered, and are this more vulnerable to damage than they would be otherwise. As a logical corollary, I tested the hypothesis that rafting colonies would compensate for their elevated vulnerability through and increase in worker defensiveness. I measured defensiveness using the amount of venom workers deliver per sting (venom dose) since the pain and tissue damage caused by fire ant venom (i.e., its repellency) is dose-dependent (Read *et al.*, 1978. *Toxicon* 16:361-367). Workers consistently delivered significantly higher venom doses (~ 87% higher on average) while rafting than they did defending their nests pre-flood. Mechanistically, the unusual concentration of workers during rafting may result in a concomitantly unusual concentration of alarm pheromones, and thus the increased defensiveness. Functionally, the increase in venom dose during rafting should serve to better protect the exposed colony from molestation. Previously reported observational data (Haight and Tschinkel, 2003. *Toxicon* 42:673-682) have suggested *S. invicta* workers increase their venom dose during periods of increased risk to colony assets (e.g., during the springtime production of sexuals), but the present data are the first to show such an increase experimentally. From a practical standpoint, human encounters with fire ants during flood conditions have the potential to be unusually dangerous; not only are large concentrations of workers exposed and available for defense, but they deliver significantly larger venom doses when they sting.

Isolation and Characterization of Venom Proteins of RIFA Queens

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Introduction

Queens of the red imported fire ant, *Solenopsis invicta*, produce a pheromone that induces workers to kill sexual larvae (Klobuchar and Deslippe 2002). The pheromone loses activity when extracted in organic solvents but retains full activity when extracted in buffered saline. Both fresh (1 day) and old (21 day) extracts of poison glands have also been found to be equally effective at inducing executions (Klobuchar and Deslippe 2002). Based on these findings, we hypothesized that the pheromone is proteinaceous and is spread over the queen's body via the stinger (Figs. 1-5).

To prepare for expression and testing of the function of the proteins of the queen poison sac, we addressed three main objectives:

- Screen the poison sacs of queens (*S. invicta* and *S. geminata*) for proteins.
- Sequence prominent proteins and compare them to the allergens stored in the poison sac of both *S. invicta* and *S. geminata* workers.
- Determine whether allergen antibodies of workers are immunologically similar to venom gland proteins of queens.

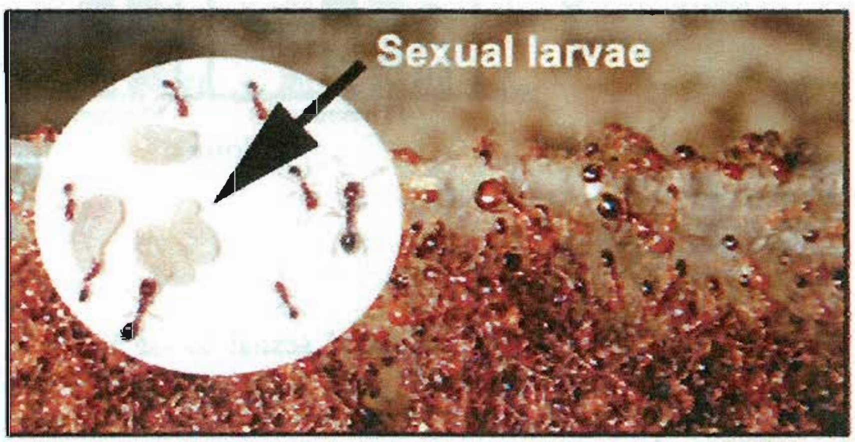

Fig. 1. Sexual larvae are energetically expensive to develop and maintain. When colonies are stressed, workers begin to cannibalize sexual larvae, perhaps to reallocate elsewhere the energy invested in the larvae. The signal to execute comes from the queen.

Materials and Methods

Partial amino acid sequences were obtained using SDS-PAGE, electroblotting and N-terminus sequencing. Full-length cDNA sequences were obtained using primers designed from previously sequenced worker allergens of *S. invicta*, RT-PCR, touchdown PCR and standard cloning techniques. Growth curves were developed by inoculating culture tubes with overnight transformed culture stocks and taking 1/10 dilution readings every 30 min for 8 h. Immunoblots were carried out by electroblotting venom sac proteins onto PVDF membrane. Detection was achieved using monoclonal antibodies reactive with denatured *Sol i* allergens II and IV and an Opti-4CN Goat-anti-Mouse kit (BioRad, Inc.).

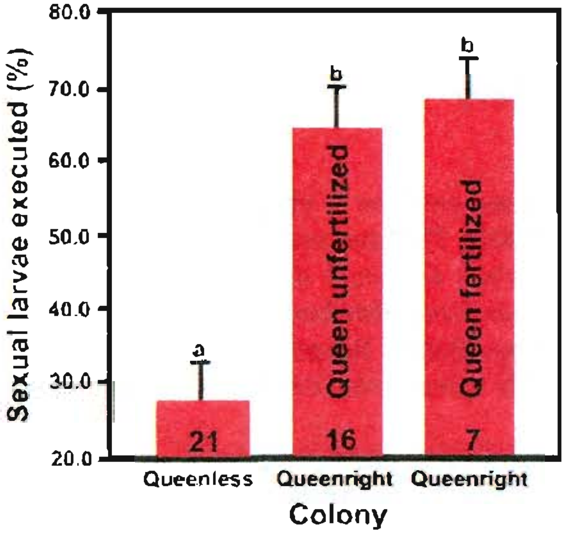

Fig. 2. Percentage of sexual larvae killed by workers after 96 h in queenless and queenright colonies. The queens in queenright colonies were either fertilized or unfertilized and differences among comparisons were significant ($F_{2,\ 41}$ = 19.82; P < 0.0001).

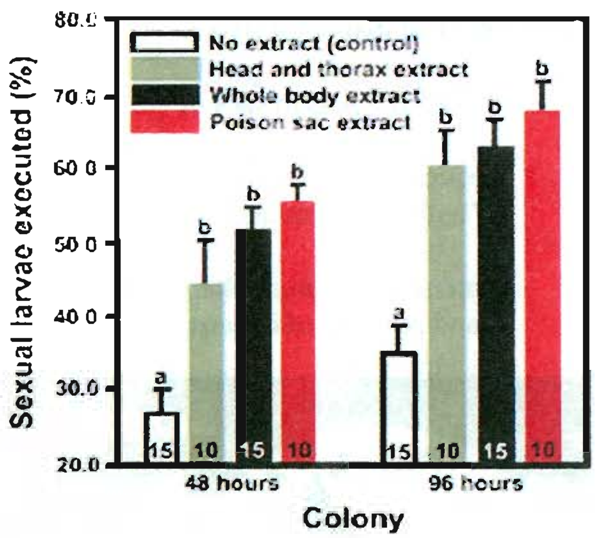

Fig. 3. Percentage of sexual larvae killed by workers in colonies receiving phosphate buffered solution (PBS) only (control), or receiving extracts of queen body parts in PBS. Differences among comparisons were significant after both 48 h ($F_{2,\ 46}$ = 13.31; P < 0.0001) and 96 h ($F_{2,46}$ = 16.71; P < 0.0001).

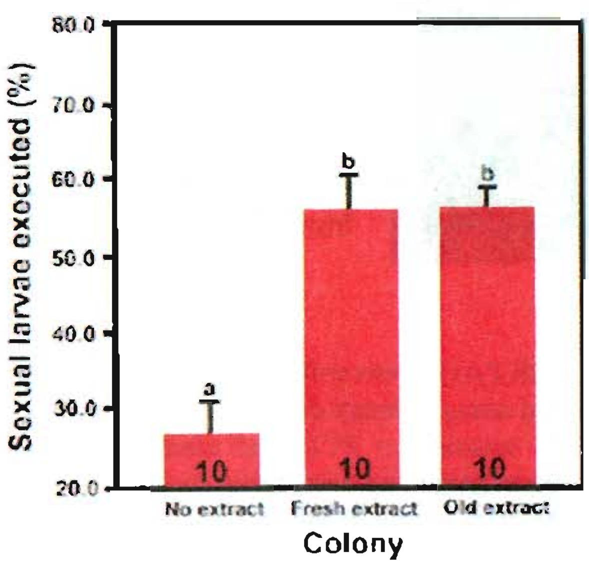

Fig. 4. Percentage of sexual larvae killed by workers after 48 h in colonies receiving PBS only (control), or receiving either fresh (1 d) or old (21 d) extracts of poison sacs of queens in PBS. Differences among comparisons were significant ($F_{2,27}$ = 29.81; P < 0.0001).

Fig. 5. In addition to venom alkaloids in an aqueous solution, the poison gland secretions of both workers and queens contain a small percentage of proteins.

Results and Discussion

a. cDNA Sequences

Prominent protein bands were isolated at 17 kDa and 28 kDa in both poison sacs and whole body rinses of alate and dealate queens using SDS-PAGE (Fig. 6). Partial protein sequences of these bands revealed that the 17 kDa band was comprised of two proteins with some similarity in sequence to the worker Sol *i* II and Sol *i* IV allergens. The 28 kDa band also showed some similarity to the Sol *i* II allergen of workers. Full cloning and comparison of translated cDNA sequences of the proteins from workers (Hoffman 1993) and queens of both *S. invicta* and *S. geminata* revealed shared amino acid identities in the sequences ranging from 72.3% to 90.8% for *Sol i* II and 85.1% to 97.4% for *Sol i* IV (Fig. 7).

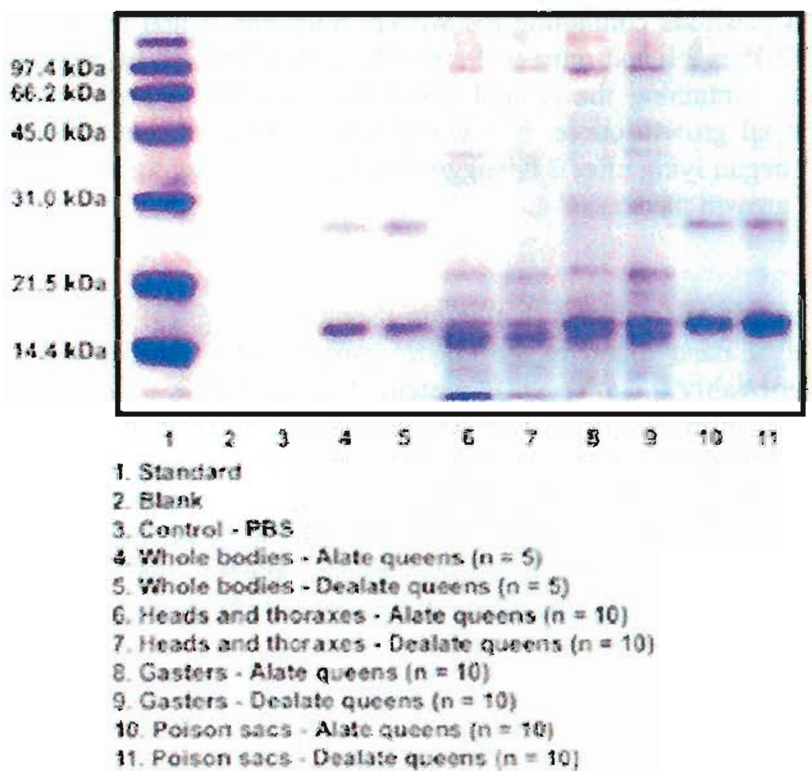

Fig. 6. Protein bands of alate and dealate queens of *S. invicta* on a 15% Tris-HCl gel.

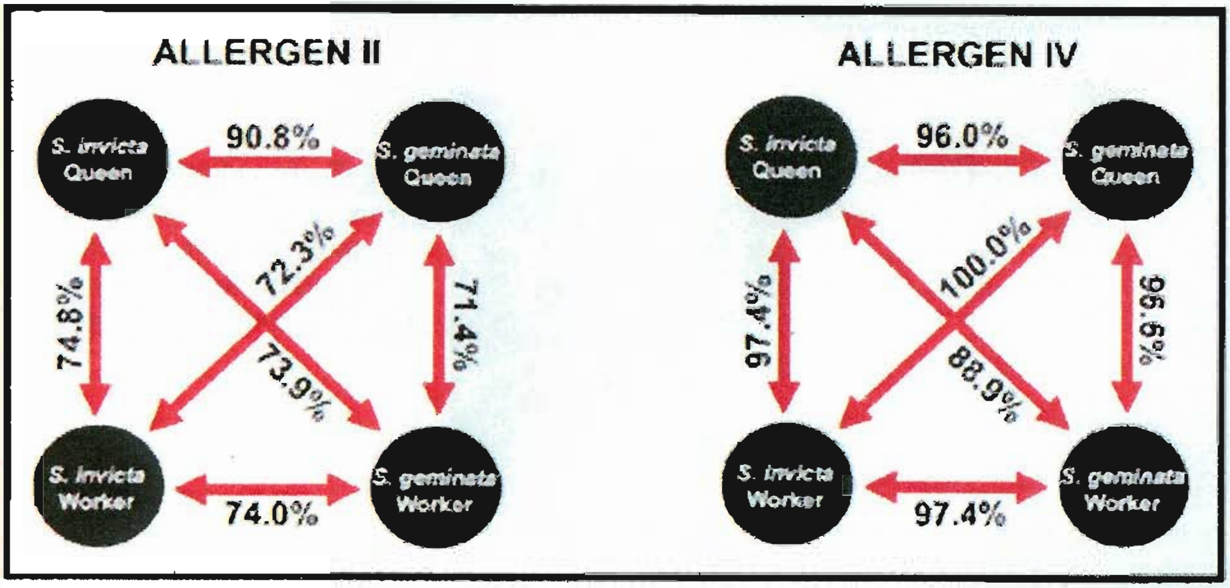

Fig. 7. Summary of allergen II and allergen IV shared amino acid identity in comparisons between workers and queens in both *S. invicta* and *S. geminata*. For allergen IV, only the comparison of one of two isoforms is included, the comparison with the greatest shared identity.

b. Bactericidal Properties

Transformed bacteria containing cDNA of worker allergens II and IV were difficult to cultivate. Consequently, optical density curves were used to help understand the growth of the transformed bacteria. Bacteria transformed with plasmids containing the worker allergens II and IV cDNA reached an absorbance of about 0.02 after 2 h and remained static thereafter (Fig. 8). In contrast, bacteria transformed with plasmids containing the related queen protein cDNAs grew much better and followed a typical bacterial growth curve. It was repeatedly observed that bacteria containing worker allergens cDNA began lying after 2 h, suggesting toxicity of low level protein expression of those allergens in the growth media.

c. Immunoreactions

In immunoblots, monoclonal antibodies of the *Sol i* II allergen (Schmidt *et al.* 1996) reacted strongly with a low molecular weight band found in the venom samples of *S. invicta* and *S. geminata* workers. The band is presumably the allergen II protein. The antibodies also reacted slightly with protein bands of molecular masses of 50 kDa and 240 kDa. The antibody did not react with poison sac samples from alate queens, however. The membrane containing the electroblotted proteins was duplicated with one half being stained with Coomassie blue stain and the other half used to perform the immunoblot. The stained half contained the 14 kD band found in the queens indicating that the half used for the immunoblot had ample protein to react with the antibody. The antibody also reacted with a 50 kDa band found in the whole body rinse sample from the queens (Fig. 9).

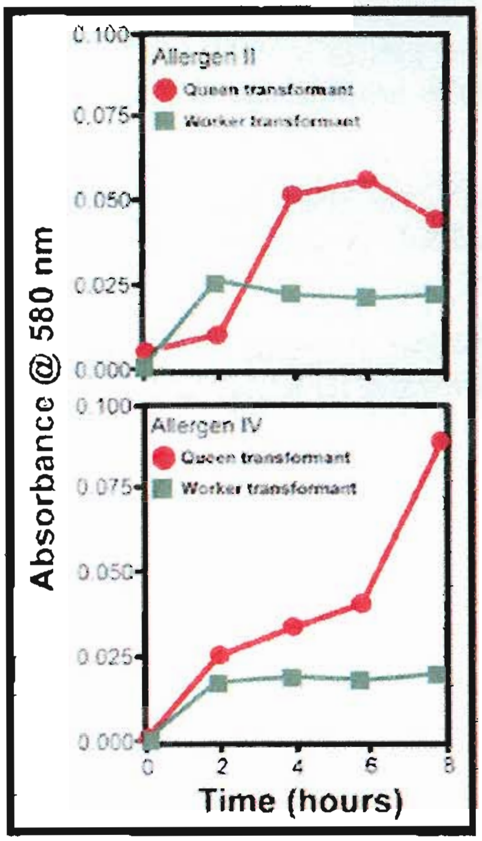

Fig. 8. Growth curves of queen and worker transformants of allergens II and IV of *S. invicta*. Transformed bacteria containing the queen protein cDNAs grew better than transformed bacteria containing the worker allergen cDNAs. The results suggest that the queen proteins are less bactericidal than the homologous worker proteins.

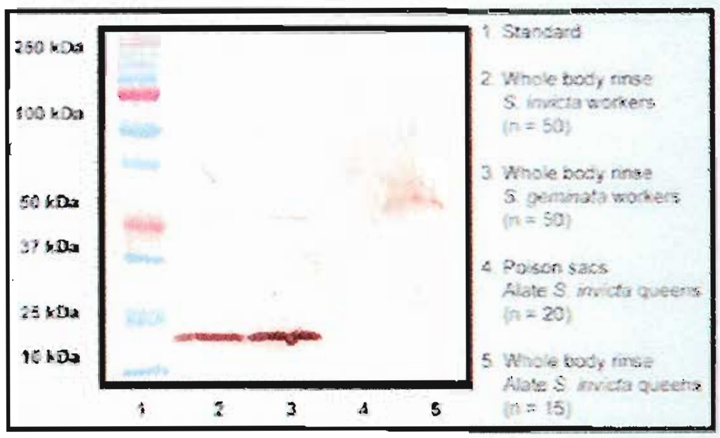

Fig. 9. Immunoblot using monoclonal mouse antibodies against Sol *i* allergen II.

Conclusions

- The proteins found in the queen poison sac differ substantially in amino acid sequences from the same sized allergen proteins found in worker venom sacs for both *S. invicta* and *S. geminata*. Differences were greater in comparisons to *Sol i* II than in comparisons to *Sol i* IV.

- Population growth of transformed bacteria containing the worker allergen cDNA differs from population growth of transformed bacteria containing the related queen protein cDNA. The growth curves imply that the 17 kDa worker allergens are more bactericidal than the 17 kDa queen proteins.

- Immunoblots using antiserum developed against the worker allergens react with the proteins found in the venom sac of workers but not with the proteins found in the venom sac of queens.

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Comparison of Glutathione *S*-transferase Activity from the Red Imported Fire Ant, *Solenopisis invicta*, and Argentine Ant, *Linepithema humile* (Mayr)

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INTRODUCTION

Glutathione *S*-transferases (GSTs) catalyze conjugations by facilitating the nucleophilic attack of the sulfhydryl group of endogenous reduced glutathione on electrophilic centers of a vast range of xenobiotic compounds, including insecticides. At least nine classes of GSTs have been identified in mammals, eight cytosolic and one microsomal class. In insects, they have attracted attention because of their involvement in insecticide resistance (Valles et al 2003). GST-based resistance to insecticides is facilitated by increased level of expression of one or more GSTs (Konanz and Nauen 2004).

Red imported fire ants (RIFA), *Solenopsis invicta* (Buren), are distributed throughout the states of Alabama, Mississippi, Louisiana, Georgia, Florida, South Carolina, and a large portion of Texas, as well as the more southern counties of North Carolina, Tennessee, Arkansas and Oklahoma. There are also some isolated areas of RIFA infestation in Arizona and California.

In its introduced range, argentine ants, *Linepithema humile* (Mayr), are important urban and agricultural pests that have had dramatic effects on terrestrial ecosystems by displacing native ants and other arthropods (Holway 1998, Silverman and Roulston 2001). This ant produces large, multiple-queen colonies that lack clear boundaries because of a general absence of intraspecific aggression, which contributes to its invasive success. Also, the large, diffuse colony structure of *L. humile* makes nests difficult to locate and treat (Silverman and Roulston 2001).

- The objective of this study was to compare GST activities between fire ants and argentine ants. We measured the effects of temperature, protein content on GST activity from different ants, and effects of some insecticides on GST activity from RIFA.

MATERIALS AND METHODS

1.1 Collecting and maintaining the ants: Colonies of *S. invicta*, were collected from the lawn on the Louisiana State University. Among them, colony 2B and 1C were approximately 100 meters apart. Workers of *L. humile*, were a gift from Dr. Michael K. Rust from University of California, Riverside, and another An additional colony of *L. humile* was collected from Toledo Bend, Louisiana.

1.2 Enzyme assay: Activities of GST were measured using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates with slight modifications according to Ottea et al (1997) in 96-well microplates. Abdomens from worker ants chosen randomly from rearing condoes were used as enzyme source. Buffers used included: sodium phosphate buffer (PB, 0.1 M, pH 6.4-8.0), Tris-HCl (Tris, 50 mM, pH 7.2-8.8), ammonia-ammonium chloride (AMCl, I=0.2, pH 8.2-10.0). All experiments were performed in triplicate and repeated three times.

1.3 Chemical treatment: Experiments were conducted in small plastic boxes with three cells (Fig.1). Harborage was places into the left cell for ants. The middle cell used for the chemical treatment area. Ants were provisioned with freshly killed crickets as food iin the right cell. There are holes on the virtical partitions. Stock solution of chemicals were prepared in acetone, and then diluted with distilled water to corresponding to the LC25, LC50, and LC70 for deltamethrin (99% purity, Sigma) and fipronil (technical grade, 89% purity; Aventis). The final volume of acetone was less than 1%. Rectangular filter paper (Whatman No.4, 5.4 X 7.9 cm) was dipped into chemical solution for 5s, and then placed on the bottom of the middle cell. Water mixed with acetone served as a blank. Boxes were held in a large plastic container in insect rearing room. At days 1 and day 14, workers were removed for enzyme assay.

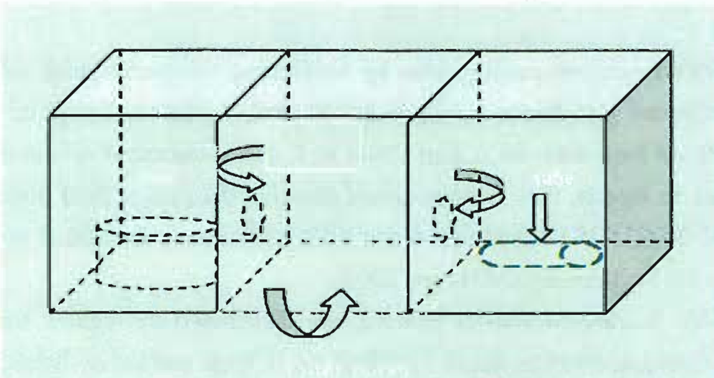

Figure 1. Diagram of test apparatus

1.4 Statistic analysis: Enzyme activities were compared between treatments and controls using a one way analysis of variance ANOVA) and Duncan's Multiple Range Test (DMRT, $P<0.05$).

RESULTS AND DISSCUSION

Results from preliminary studies suggest that pH 8.0 sodium phosphate buffer is optimal for measuring GST activity in *S. invicta* ; however, in assays with *L. humile*, activities were greatest in either Tris-HCl, pH 8.8 and AMCl, pH 10 (data not shown). For both species, the best activity was measured at 25□ for any kind of buffer solution tested (Table 1).

Table 1 Effect of temperature on GST activity

Temperature	GST activity in fire ants		GST activity in argentine ants	
(°C)	PB pH 8.0	AMCl pH 9.4	Tris pH 8.8	AMCl pH 10
20	419.20	214.67	682.62	614.23
25	557.96	344.27	1189.5	2244.28
27	428.18	129.57	376.35	625.38
30	406.19	90.94	364.80	225.99
35	247.88	28.78	64.52	67.74
37	371.80	103.56	80.40	0
40	395.07	0.48	260.01	150.67

GST activity unit is $nmol \cdot min^{-1} \cdot mg^{-1}$ protein

Differences in protein/activity relationships were measured in assays with the two insects species (Figure 2). GST activities in both pH 8.0 sodium phosphate buffer in fire ants and pH 8.8 Tris-HCl buffer in argentine ants were linear within the range of protein concentrations examined (0-5 μg) (Fig. 2).

We also found that GST activity differs among colonies. GST activity of argentine ants from Toledo Bend was 7.5-fold higher than that from California (Table 3). It is not clear whether this difference is clue to regional diversity, different background of insecticide used or others.

Under defined conditions, effects of deltamethrin and fipronil on GST activities in fire ants were determined. Compared with that in controls, GST activity was significantly higher in ants treated with both fipronil and deltamethrin at Day 1. At 14 days after treatment, there were no significant differences between most chemical treatments and control on Day 14 except treatments at LC50 of fipronil and LC70 of deltamethrin (Fig.3). These imply that GST may not be a detoxification enzyme toward deltamethrin and fipronil in fire ants.

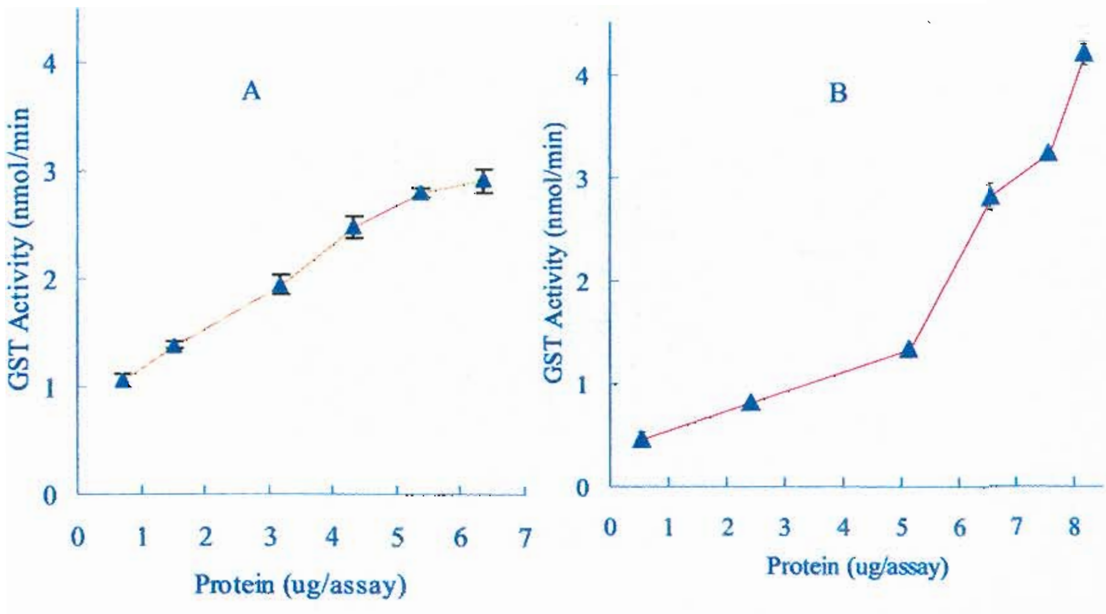

Figure 2 Protein dependence of GST activity in argentine ants (A) and fire ants (B)

Table 2 GST activities in fire ants from different colony

Colony	PB pH 8.0	AMCl pH 9.4
1C	341.06±19.87 a	0 b
2B	337.79±5.98 a	782.64±187.19 a

GST activity unit is nmol·min-1·mg-1 protein

Means followed by the different letter in the same column are significantly different (DMRT, P<0.05) (The same below)

Table 3 GST activities in argentine ants from different colony

Colony	Tris-HCl pH 8.8	AMCl pH 10
California	0.221±0.081 b	12.661±0.647 b
Toledo Bend	1.662±0.446 a	15.337±0.631 a

GST activity unit is mmol·min-1·mg-1 protein

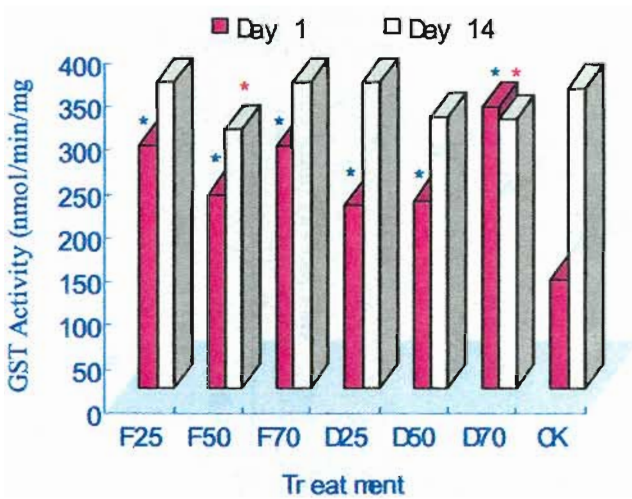

Fig. 3 Effects of deltamethrin (D25, D50, and D70) and fipronil (F25, F50, and F70) on GST activity in fire ants

* Aster indicated the difference between treatment and CK differ significantly by DMRT (P<0.05)

SUMMARY

Conditions for measuring GST activities from fire ants, *S. invicta*, and argentine ants, *L. humile* (Mayr), were optimized, and were similar between the two species. GST activities differed from kind to kind, colony to colony, and may not be a detoxification enzyme toward deltamethrin and fipronil in fire ants.

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Molecular identification of the Red Imported Fire Ant *Solenopsis invicta* imported into Chinese Mainland

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Abstract The Red Imported Fire Ant (RIFA), *Solenopsis invicta*, an important global quarantine species, was reported invading and diffusing in Chinese Mainland and Hong Kong, and leading to increasing concern of society recently, In this paper, the RFLP patterns and partial sequences of rDNA ITS from the specimens of *S. invicta* have been determined for the first time and compared with those of *Solenopsis* sp. and *Solenopsis geminata* which were collected respectively from Wuchuan County and Guangzhou in Guangdong Province of China.

Genomic DNA was extracted from a single ant of each species. The ITS regions of ribosomal DNA of the three samples were amplified by polymerase chain reaction (PCR) respectively with eukaryote-universal primers. From each sample, a specific product of amplification of rDNA ITS regions (about 2.5kb in length) was obtained, which was longer than that of other known insects. The rDNA ITS libraries were constructed and the positive clones were screened by RFLP analysis with a DNA restriction endonuclease *SalI*. From the results of RFLP analysis, three distinctive RFLP patterns (termed I, II, III) determined in the libraries from *Solenopsis* sp. were identical to the three RFLP patterns in the libraries from specimens of *S. invicta*, which collected from U.S.A. However, there were only two types of RFLP patterns (4I and 4II) in the libraries from *S. geminata*. Hereinto the pattern 4I is similar to the RFLP pattern II. Nevertheless pattern 4II is completely different from all the other patterns. Based on the statistics, the percentage of the clones of RFLP pattern I, II, III was 53%, 6% and 41% in *S.* sp. ITS libraries and 67%, 8% and 25% in *S. invicta* ITS libraries, respectively. The pattern 4I and 4II represent 40% and 60% of total clones in the *S. geminata* ITS libraries, respectively. Noticeably, high sequence similarity (>99%) of rDNA ITS between *S. invicta* and *S.* sp. were observed when compared the sequences from the RFLP type I and III, which were the most two abundant RFLP types in both species. To the contrary, *S. geminata* was much different from above two species. The sequence similarity between *S. geminata* and *S. invicta* or *S.* sp. was only 96.2%-97%.

The analysis resoults conformed that *Solenopsis* sp. from Wuchuan county is as the RIFA, *S. invicta*, introduced into Chinese Mainland. The rDNA ITS regions, therefore, can serve as a suitable molecular index for the identification and systematic analysis of RIFA and related species.

Key words: *Solenopsis invicta, Solenopsis geminata,* molecular identification, rDNA ITS RFLP

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Abstract: The Development of a Real-Time Detector for Fire Ant Mounds on Pasture Ground.

Imported fire ants on pastures are a problem that is managed primarily by the use of insecticidal baits. In areas of fire ant management where mound density is maintained at low levels, an automatic detector of fire ant mounds can be a viable means of facilitating site specific management of fire ants and may lead to a more economical method of fire ant management.

Broadcast and individual mound applications of hydromethylnon bait (Amdro) were compared and found to be statistically equal in reduction of active fire ant mounds and foraging fire ants. The effect of these application methods on native ants populations was evaluated by recording numbers of the little black ant, *Monomorium Minimum* because it was the only native species collected in sufficient numbers for analysis. Treatments included broadcast bait (2 lb formulated bait per acre) applied with a 4-wheeler equipped with model GT-77 Herd seeder (Herd Seeder Ca., P.O. Box 448, Logansport, IN 46947), and individual mound treatments of 19.5 g/mound (approximately 4 tablespoons applied by hand), and untreated pasture. Plots were 125 ft wide by 350 feet long. Three 100 feet diameter circles were sampled in each plot by placing 10 hot dog baits contained in a survey trap evenly spaced in a 40 ft diameter circle within each 100 ft circle.

There was no statistical difference in fire ant control between broadcast and individual mound treatment methods. Little black ant populations were unchanged in blocks where individual mounds were treated, but were reduced to near zero in blocks where bait was broadcast indicating that individual mound treatment would be preferred in areas where native ant species are to be preserved such as in national parks and wildlife preserves.

The economics of grid-based application become attractive when the mounds are clustered such that only a few number of grids need to be treated. The computation of cost for the grid-based treatment was done by computing the cost per square foot using the base cost of $16.00/acre (the broadcast cost of 2 lb of Amdro per acre). It is obvious that the grid-base

treatment is more cost effective than broadcast treatment as long as there is a number of grids on the field that do not require treatment .

The economy of individual mound treatment using Amdro is justifiable when the number of mounds per acre is less than 47. By dividing a field into grids, particularly if mounds are not uniformly distributed throughout the field, grid-based treatment of individual mounds may be more economical than broadcast bait application. Surface thermal profiling may be used to discriminate fire ant mounds from other materials such as grass. An automated real-time fire ant mound detector was developed and is under going field testing to allow automated individual mound treatment of field sized areas. Real-time electronic detection of fire ant mounds presents an interesting opportunity for grid-based application. The occasional false positive results suggests that a courser grid may help in lumping many of the falsely detected areas into some grids, but a more accurate detection is still needed in order to facilitate finer grid experiments.

Development of a Quarantine Survey Bait-Attractant for the Red, Hybrid and Black Imported Fire Ants

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Introduction: Numerous food products have been evaluated for attraction to the Red Imported Fire Ant (RIFA). Much of this was done by Lofgren et al. in 1961 and 1964 on a large variety of vegetable and animal products. These were both single ingredient products such as oils and sugars and complex products such as cereals and baby foods. The goal of their research was the development of an attractant or bait for use with an insecticide. The goal of our research has been to develop an attractant to be used in quarantine surveys where mounds are not readily observable. Because there have been changes in food products in the last forty or so years we have repeated some of this earlier work (Lofgren et al. 1961, 1964). The best source of vegetable oils for the more limited volumes that survey baits require are those for human consumption (cooking oils) which are available at any grocery store. These also have strict production standards which the once refined oils do not. There are numerous manufactured food products and snack foods that are attractive. It also was a goal to identify a few of these for field and comparison evaluations.

The development of a bait requires that it is:

- attractive to all 3 genotypes of the Imported Fire Ant (IFA)
- attractive year round to IFA
- surveyor friendly or easy to handle by someone putting out many survey traps
- manufactured in desired size and packaged for use
- have a stable self life both in and out of packaging
- composed of known unaltered ingredients that are economical and available over time
- not be a popular food source for human observers and participants

These findings are presented here.

Methods and Materials: The laboratory test method was a standardized SIPS protocol based on Lofgren et al. (1961, 1964). Field collected IFA worker ants (adult and immature) with mound soil were collected with a small bladed shovel and placed in plastic 11.4 quart dishpans or 12 qt. sweater boxes. Each treatment was composed of 5 replications of collected ants in plastic boxes. The insides of the boxes where dusted with talcum powder to prevent escape. The ants were held for 3 to 5 days without food before testing. On the day of the test the soil was watered and a board (1"x2"x12") was placed in each box on the soil. At each end of the board a petri dish (100mm x 15mm square or round) bottom was placed. One dish contained 4 grams of the candidate product and the other was the standard bait. After a period of 24 hours the petri dish bottoms were lidded, collected and weighed. The dishes had been numbered, weighed and recorded per replication per treatment before being tested. The finished weights were subtracted from the beginning weights. These weights were then recorded and an acceptance ratio (grams candidate bait removed/grams standard bait removed) was calculated for each replication with a mean acceptance ratio calculated per treatment (Table 1).

The standard bait was peanut oil (30% by weight) mixed with pregelled corn (70% by weight). The same mix ratio was used for all the oils. The solid candidates were ground into small particles with mortar and pestle. The evaluated candidates or attractants are listed in Table 1.

Results and Discussion: The results of these evaluations are presented in Table 1. Lofgren et al. (1964) considered all evaluated foods with ratios of 0.75 and above to be acceptable. Since our adaptation of the evaluation procedure uses removal of food as the criterion for evaluation we have added some variables that Lofgren et al. (1961, 1964) did not have. These include the physical state and particle size of the food. One variable that we both faced was the physical condition of the ant colony used in each test replicate. This includes both health and population size.

Cooking Oils: These evaluations show no distinct differences among any of the cooking oils. The 2002 data for corn oil indicated a preference for corn oil but further testing showed none. The cooking oils performed as well as the once refined peanut and cotton seed oils and were much easier to obtain. The crude cottonseed oil was old but tested because it had been processed without the more modern chemical solvent techniques. This result agrees with Lofgren et al. (1964) that a crude grade of fat is not as acceptable as a refined. They also found that highly unsaturated fats and hydrogenation were not preferred by RIFA. Hydrogenation is being reduced or eliminated in present day cooking oils. Thus these cooking oils will continue to be attractive to IFA.

Commercial Products: The products for human consumption all showed attraction for the IFA. The physical properties of peanut butter, honey and mayonnaise caused these materials to have lower acceptance ratios. This is demonstrated by the results of mayonnaise mixed with pregelled corn. The lower values of Tostado Corn Chips®, pork skins and some of the others can be blamed on larger particles not ground fine enough, thus the ants could not physically remove the food particles.

The pet products presented grinding problems with a mortar and pestle. The dog and cat products did have promising results and contained noticeable oil. The aquatic pet foods were dry with much less oil in them.

Seeds or Nuts: Most of these showed some promise. They were all difficult to grind with our equipment. The whole safflower seed is well protected from IFA feeding as was pop corn. A non replicated test of plain popped popcorn demonstrated little interest from IFA.

Insect Baits or Diets: The two commercial formulations were dry formulations and as found by Lofgren et al. (1961) had limited attraction. When water was added to the insect diet the attraction was increased to an acceptable level. It is a finely ground formulation which would make it difficult to use as a field bait or attractant. The IFA Survey Bait Prototype was designed after many of these attraction evaluations were completed. It performed well in these tests as preliminary developmental work had indicated. It is formulated similar to granola bars and molded into a small dome-shaped tablet. Each tablet or bait is sized for a single trap. It is composed of pregelled corn, vegetable oil, several protein sources and several sugars.

Conclusions: Numerous food products are attractive to IFA as noted in this study. Most oils available in grocery stores are attractive to all genotypes of IFA leading to the conclusion that prepared food products containing higher percentages of vegetable oils would also be attractive to IFA. The pet foods tested in this trial were generally too dry for IFA and the mature seeds and nuts were protected from IFA feeding and needed to be ground in order to be good attractants or baits.

The products from the laboratory testing that were consistent and easy to handle were then selected for field evaluation. These are the ones tested for the Black IFA (Table 1). These include 5 commercial products, the Survey Bait Prototype and the peanut oil-pregelled corn as a standard. Two of the commercial products were selected based on their use in the California RIFA Eradication Program; the Spam® and Frito's®. The field evaluations will be reported when completed.

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Table 1: Laboratory Evaluation of Food Products for Attracting the 3 Genotypes of Imported Fire Ants

Cooking Oils in pregelled corn

Type of Oil	Acceptance Ratio of candidate attractants in IFA genotypes		
	RED	HYBRID	BLACK
Canola	1.00	0.98	1.00
Corn (2002)	1.79	2.49	----
Corn (2003-2004)	----	1.02	1.00
Corn vs Soybean	1.06	1.00	1.00
Olive	1.01	1.15	1.00
Safflower	1.00	1.00	----
Soybean	1.00	1.14	1.00
Sunflower	1.00	1.06	1.00
Lard	1.00	1.00	1.32
Mayonnaise	1.00	1.44	1.14
Soybean (27%) + Karo Syrup (3%)	----	1.00	----

Other Oils in pregelled corn

Type of Oil	Acceptance Ratio of candidate attractants in IFA genotypes		
	RED	HYBRID	BLACK
Cod Liver	0.00	0.59	----
Cottonseed-once refined	----	1.00	----
Cottonseed-raw	----	0.44	----
Peanut-once refined	----	1.00	----

Commercial Products used from package no mixing

Product	Acceptance Ratio of candidate attractants in IFA genotypes

	RED	HYBRID	BLACK
Chezit Crackers®	0.42	0.74	----
Frito's Corn Chips®	0.99	1.00	0.92
Honey	0.14	0.19	----
Lay's Potato Chips®	1.32	0.91	0.85
Mayonnaise	0.39	0.44	----
Peanut Butter	0.70	0.78	----
Pecan Sandies®	1.19	0.86	1.17
Pork Skins	0.72	0.99	----
Ritz Crackers®	1.00	0.97	0.88
SPAM®	1.00	0.84	1.49
Tostado Corn Chips®	0.34	0.96	----
Vienna Sausage®	1.00	1.00	----

Pet Foods

Product	Acceptance Ratio of candidate attractants in IFA genotypes		
	RED	HYBRID	BLACK
Cat Chow®	0.33	1.00	----
Dog Treats	0.50	0.84	----
Fish Food	0.66	0.20	----
Puppy Chow®	0.34	1.00	----
Salamander Food	0.36	0.10	----
Turtle Food	0.48	0.59	----

Seeds or Nuts

Product	Acceptance Ratio of candidate attractants in IFA genotypes		
	RED	HYBRID	BLACK
Almonds	0.73	0.98	----
Chinese Pine Nuts	0.87	0.02	----

English Walnuts	0.72	1.00	----
Hazel Nuts	0.68	0.27	----
Macadamia Nuts	0.40	0.78	----
Safflower-Whole	0.08	0.00	----
Safflower-Ground	----	1.00	----
Sunflower-Ground	0.97	0.88	----

Insect Baits or Diets

Product	Acceptance Ratio of candidate attractants in IFA genotypes		
	RED	**HYBRID**	**BLACK**
APHIS IFA Survey Bait Prototype	1.28	1.02	1.00
Artificial Media for Rearing (AMR*) Entomophages®-Dry	----	0.35	----
AMR Entomophages®-Moist	----	0.99	----
Tast-E-Bait®**	0.53	0.83	----

* USDA REE ARS MSA. U.S. Patent #5,834,177
** Bakery based carrier/attractant for some commercial IFA toxic baits

Impact of *Solenopsis invicta* on arthropod diversity at two sites in southeastern Oklahoma

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Introduction

Red Imported Fire Ant, *Solenopsis invicta* was accidentally introduced into the United States from South America during the 1930s. Since then, the Red Imported Fire Ants (RIFA) has gradually spread and infested more than 260 million acres, from North Carolina to Texas. RIFA expanded its habitat northward and was first reported from Oklahoma in the mid-1980s. *S. invicta* is more abundant in the United States than in its natural habitats in South America. The ecological dominance of RIFA in the introduced habitats can be attributed to the lack of natural enemies in the introduced habitats. The impact of *S. invicta* on native ant species and other arthropod communities has been greatly discussed during the last two to three decades, but not resolved. Here, we discuss the outcome of the research conducted from spring to fall 2003 and 2004, at Adam and Bowles Ranches, Bryan Co., OK, as part of the Area-wide Fire Ant Suppression Program.

Methods

The two study sites, Adam and Bowles Ranches (Fig 1) have ten plots each in the 150-acre treatment area and in the peripheral control area, 9 and 12 plots, respectively. All plots are of 1/8th acre in size and divided into four quarters from the center based on the four directions. Pitfall traps were set at 14 ft radius from the center, on all four directions, in May (Pre-treatment) and September (90-days after treatment) 2003 and 2004. The traps were collected after 48 hours, sorted in the laboratory and identified. All ant samples were identified to genus/species level. Other insects and invertebrates were identified to family level and categorized as morphospecies. In June 2003 and 2004, 150 acres (areas delineated by blue and yellow lines in the Fig 1.) were treated with insecticides at both sites. Data on morphospecies was statistically analyzed using Mann-Whitney U-test.

Results and Discussion

The pretreatment studies in 2003 indicated that the mean number of *S. invicta* per plot was lesser in the plots within the treatment area compared to the untreated area (7.0 and 13.7 in the treatment plots and 21.1 and 61.5 in the untreated plots of Adam and Bowles Ranches, respectively Table 1). Similarly, 90 days after treatment, the mean number of RIFA per plot was low in treated plots of Adam and Bowles Ranch compared to the untreated plots in the respective sites.

The trend was similar in treatment and non-treatment areas of both ranches, in 2004. In Bowles Ranch, the number of RIFA caught in the pitfall trap reduced by half in the treatment area during the 90-day post-treatment study (Table 2).

The mean number of morphospecies per trap was significantly higher in the treatment plots compared to the untreated plots of both sites, before Insecticide treatment (Fig 2a; Mann-Whitney U-test: Adam Ranch at $p<0.005$ and Bowles Ranch at $p<0.01$). Post-treatment (90-days after treatment) analyses indicated that the number of morphospecies was not significantly different between treated and untreated plots at Adam Ranch (Fig 2b; Mann-Whitney U-test: at $p>0.05$), due to a reduction in the number of morphospecies in the treated plots. The treated plots at Bowles Ranch, contained significantly more number of morphospecies than the untreated plots in the post-treatment pitfall traps (Fig 2b; Mann-Whitney U-test: at $p<0.05$).

In 2004, the mean number of morphospecies per trap was not significantly different between treated and untreated plots of both ranches (Fig 2c&d). At Adam Ranch, the mean number of morphospecies gradually increased in the treatment area, though it was not significantly different from the increase in the untreated plots. It is interesting to note that this site was not treated at all in 2004. The pretreatment and post-treatment evaluations at Bowles Ranch indicated a higher mean number of morphospecies per pitfall trap in the treatment areas, though not significantly higher than the non-treatment areas.

2003 and 2004: Adam and Bowles Ranches, collectively, had 11 species of ant belonging to four subfamilies (Table 2). *Crematogaster sp., Pogonomyrmex sp.* and *Neivamyrmex sp.* were found in one trap each in 2003, while the other species were trapped more frequently. In 2004, however, the latter two species were not caught in the traps, but *Crematogaster sp.* was found more often. Amongst the morphospecies trapped at both sites, coleopterans were the most common group of insect (Table 3). Beetles (Coleoptera) from 18 families, flies (Diptera) from 15 families and wasps (Hymenoptera) from 10 families and one superfamily were collected from the two sites.

Conclusions

The 2003 data from Bowles Ranch indicated that insecticide treatment has no significant effect on insect species other than RIFA, and that reduction in RIFA may actually result in the maintenance of species diversity. However, the results from the 2004 study are not conclusive due to factors like overall low density of RIFA throughout the year, as compared to 2003 densities. However, long-term observations would enable better analysis of the patterns and to understand the interspecific interactions along with the associated ecological determinants conclusively.

Table 1. Effect of insecticide treatment on RIFA collected in pitfall traps, in 2003

Sites	PRE-TREATMENT (May 2003)		POST-TREATMENT (Sept 2003)	
	Treated plots	Untreated plots	Treated plots	Untreated plots
	Mean RIFA/plot (Mean ± S.D)			
Adam Ranch	7.0±7.2 n=40	21.1±17.19 n=40	22.8±39.88 n=40	104.11±76.02 n=36
Bowles Ranch	13.7±26.17 n=36	60.1±78.98 n=36	13.4±18.11 n=40	184.33±154.53 n=48

Table 2. Effect of insecticide treatment on RIFA collected in pitfall traps, in 2004

Sites	PRE-TREATMENT (May 2004)		POST-TREATMENT (Sept 2004)	
	Treated plots	Untreated plots	Treated plots	Untreated plots
	Mean RIFA/plot (Mean ± S.D)			
Adam Ranch	0.4±0.84 (n=40)	19.67±19.72 (n=36)	4.4±3.31 (n=40)	66.0±49.63 (n=36)
Bowles Ranch	7.2±8.82 (n=40)	26.92±36.62 (n=48)	3.5±3.75 (n=40)	39.42±49.59 (n=48)

Table 3. Ant species identified from the pitfall traps

Order: Hymenoptera Family: Formicidae	
Subfamily: Ponerinae *Ponera sp.* Subfamily: Formicinae *Paratrechina sp.* Subfamily: Dolichoderinae *Forelius sp.* *Tapinoma sp.*	Subfamily: Myrmicinae *Crematogaster sp.* *Monomorium minimum* *Neivamyrmex sp.* *Pheidole sp.* *Pogonomyrmex sp.* *Solenopsis molesta* *S. invicta*

Table 4. List of morphospecies trapped in the pitfall traps

Order	Family
Coleoptera	Anthicidae, Anobiidae, Bruchidae, Carabidae, Chrysomelidae, Coccinellidae, Cryptophagidae, Cucujidae, Curculionidae, Elateridae, Histeridae, Latridiidae, Phalacridae, Rhizophagidae, Scarabaeidae, Scydmaenidae, Silvanidae, Staphylinidae.
Diptera	Bibionidae, Bombyliidae, Calliphoridae, Cecidomyiidae, Chironomidae, Culicidae, Dolichopodidae, Drosophilidae, Muscidae, Scathophagidae, Sciariidae, Sepsidae, Stratiomyidae (pupae), Tabanidae, Tachinidae, Tipulidae.
Hymenoptera	Braconidae, Chalcidoidae (Superfamily), Diapriidae, Eumenidae, Ichneumonidae, Mutillidae, Pompilidae, Scelionidae, Tiphiidae (Subfamily – Methochinae), Vespidae.
Homoptera	Aphididae, Cicadellidae, Delphacidae
Hemiptera	Anthocoreidae, Corimelaenidae, Cydnidae, Lygaeidae, Miridae, Pyrrhocoreidae.
Orthoptera	Acrididae, Gryllidae, Gryllacrididae.
Other insect groups	Blattaria – Blatellidae, Thysanoptera – Terebrantia (Suborder), Collembola – Symphypleona and Arthropleona (Suborders), Diplura – Japygidae (Suborder), Protura, Lepidoptera - microlepidopteran moth.
Other invertebrates: Araneae, Acari, Diplopoda, Isopoda, Mollusca and Annelida	

Fig 1. Mean morphospecies per pitfall trap (2003)

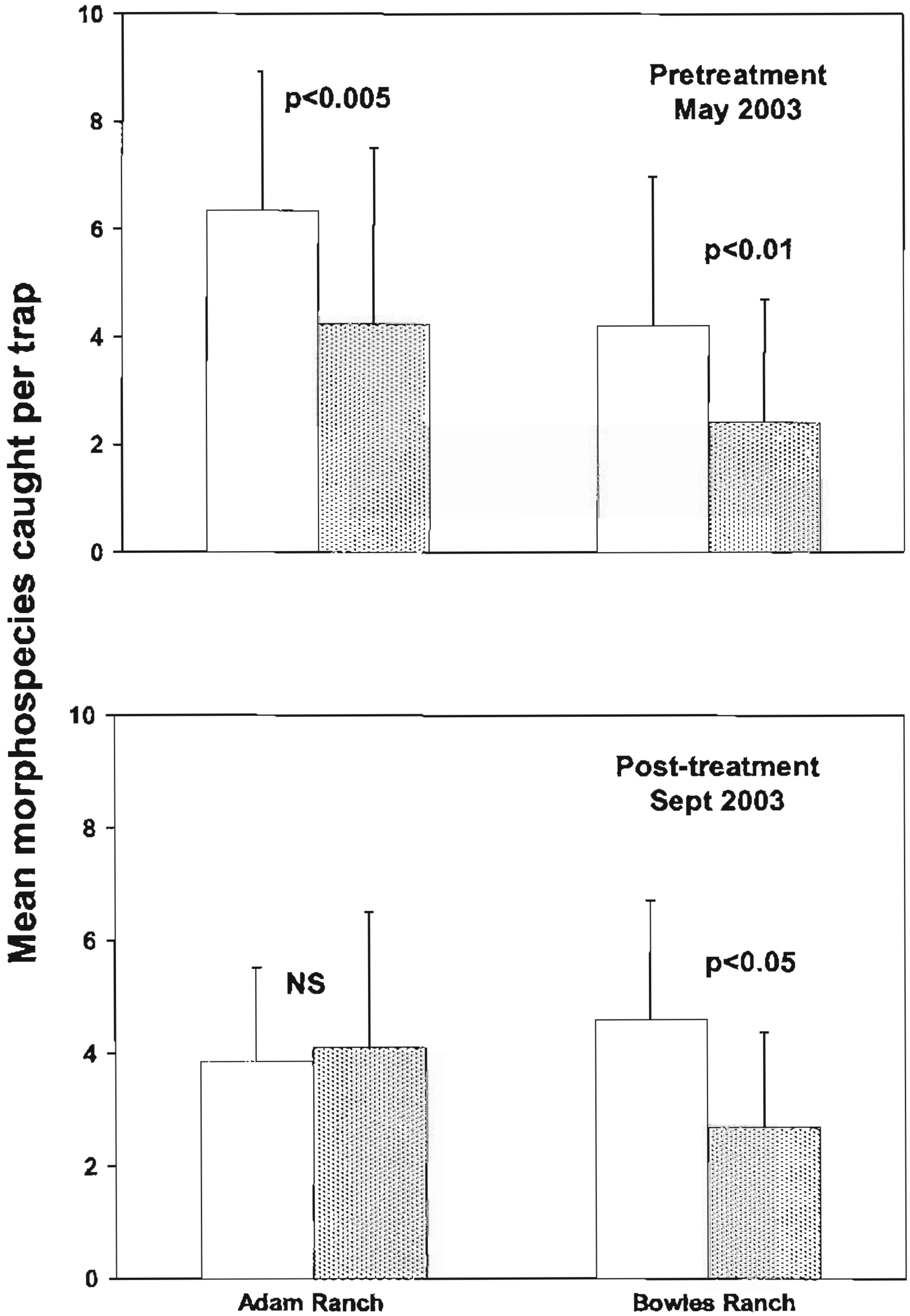
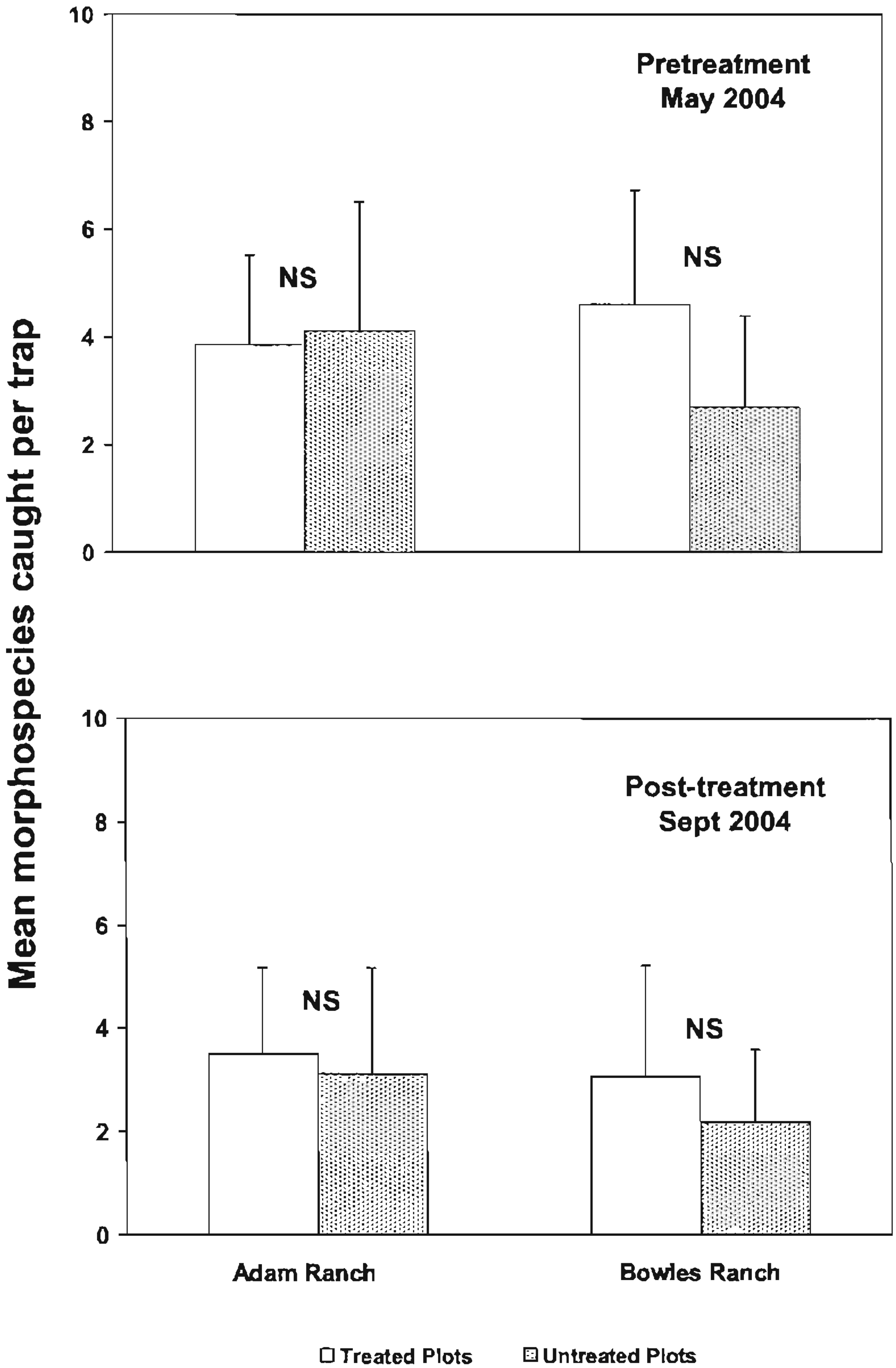


Fig 2. Mean morphospecies per pitfall trap (2004)



EFFECT OF FIRE ANT DIGGING BEHAVIOR ON EFFICACY OF FIPRONIL AGAINST WORKERS: RESULTS OF A LABORATORY STUDY

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ABSTRACT

The effect of the digging behavior of fire ant workers on the efficacy of fipronil was investigated using digging bioassays. Workers excavated fipronil treated sand in 99.5% cases at concentration level up to 10.0 ppm. In no-choice bioassays at 1.5 and 2.0 ppm, workers from a less sensitive colony had significantly higher mortality rates than those from a more sensitive colony, which could be due to the significantly higher digging activity of the less sensitive colony. In two-choice bioassays where untreated sand was also available, at 1.0 and 10.0 ppm, mortalities were positively correlated to digging effort in treated sand; however, such correlation was significant only at 1.0 ppm level.

INTRODUCTION

Digging is an intrinsic behavior of many ant species. It is essential not only in building, enlarging, and repairing nests, but also in constructing forage tunnels. The nest provides shelter to all colony members and the extensive tunnel system gives foragers protection when they are foraging outside the nest. Indeed, digging behavior is critical to ant colony development. Although it has long been a subject of research (Sudd, 1975;

Mikheyev and Tschinkel, 2004), digging behavior has not been investigated in the context of ant control. One potential scenario, in which digging behavior would have positive impact on fire ant control, is individual mound treatment using contact insecticides such as drenches. In such treatments, contact between ants and insecticide is required for treatment to be effective. Digging behavior would increase the chance for ants to come in contact with the insecticide to improve the efficacy of the insecticide. The objective of this study was to investigate the effect of fire ant digging behavior on the efficacy of fipronil incorporated into the sand.

METHODS

LC_{50}s of Fipronil for Fire Ant Workers. The LC_{50}s were determined by using 24 h mortality of 50 workers in a Petri dish with 40g fipronil treated sand. The moisture content of the sand was 8%. The inside wall of the Petri dish was coated with Fluon to ensure all ants contacted the sand. Tested concentrations of fipronil included 0.00, 0.05, 0.10, 0.50, 1.00, 1.50 and 2.00 ppm. Two colonies (A and B) were tested. Trimmed Spearman-Karber method was used to estimate LC_{50}s.

No-Choice Digging Bioassay. The bioassay apparatus is shown in Fig. 1. Seven concentrations of fipronil were tested, including 0.00, 0.05, 0.10, 0.50,1.00, 1.50, and 2.00 ppm. A mean (± SD) 34.75 g (± 0.73 g) sand was added into each vial. One hundred fire ant workers were introduced into the Petri dish. After 24 h, the sand in each Petri dish was collected and weighed. Colonies A and B were used for this experiment. There were 5 replicates for each concentration. A t-test (critical *P*-value = 0.05) was used to compare the mean amount of sand removed and mortality for each concentration between the two colonies.

Two-choice Digging Bioassay. The bioassay apparatus is shown in Fig 1. Fipronil treated sand was placed in treatment vials and untreated sand in control vials. One hundred fire ant workers were introduced into the home dish. Tested concentrations of fipronil were 0.10, 1.00, and 10.00 ppm. After 24 h, the sand in each dish was collected and weighed. If there were sand in the home dish, then the sand in each vial was collected and the amount of sand removed was calculated from the weight difference of sand in the vial before and after the digging experiment. Two colonies (C and D) were used for this experiment. For each colony, there were 10 replicates for each concentration. A paired t-test (critical *P*-value = 0.05) was used to compare the mean amount of sand removed in treatment vials with that in control vials for each concentration. At each concentration, Pearson correlation coefficient between the amounts of sand removed from treated vials and ant mortalities was calculated.

RESULTS AND DISCUSSION

Generally, workers from colony A were more sensitive to fipronil than those from colony B (Fig. 2). For both colonies, mortality rates increased dramatically with increase in fipronil concentration between 0.10 and 1.0 ppm. The LC_{50} was 0.24 ppm for colony A and 0.45 ppm for colony B. In no-choice bioassays, workers dug all fipronil treated sand; however, the amount of sand removed declined as fipronil concentration increased. Colony B removed significantly higher amount of fipronil treated sand at all concentrations than colony A (Fig. 3A). At 1.5 and 2.0 ppm, workers from the less sensitive colony B, had significantly higher mortalities than those from colony A, which might be attributed to the higher digging effort of workers from colony B (Fig 3B). In two-choice digging bioassays, workers dug into the fipronil treated sand in 59 of 60 cases, even at 10.0 ppm (Fig. 4). At 1.0 and 10.0 ppm, worker mortalities were positively correlated to digging effort for both colonies; however, such correlation was significant only at 1.0 ppm (Fig. 5). At 1.0 ppm, the correlation coefficient was 0.85 ($P = 0.002$) for colony C and 0.95 ($P < 0.0001$) for colony D. At 10.0 ppm, the correlation coefficient was 0.55 ($P = 0.10$) for colony C and 0.25 ($P = 0.48$) for colony D. At 0.1 ppm, the correlation coefficient was 0.12 ($P = 0.75$) for colony C and -0.45 ($P = 0.18$) for colony D. These suggested that digging did affect mortality; however, such effect was concentration dependent. Individual mound treatment is a common practice in fire ant management (Williams and Lofgren 1983). The implication of this study is that insecticide efficacy in mound treatments may be improved by enhancing ant digging behavior.

ACKNOWLEDGMENTS

I thank Douglas A. Streett and M. Guadalupe Rojas, USDA-ARS, Stoneville, MS, for providing valuable comments on this manuscript, and Ling Xiao Zhang and Steve Kyei-Boahen, Delta Research and Extension Center, Stoneville, MS for critical reviews of the manuscript. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

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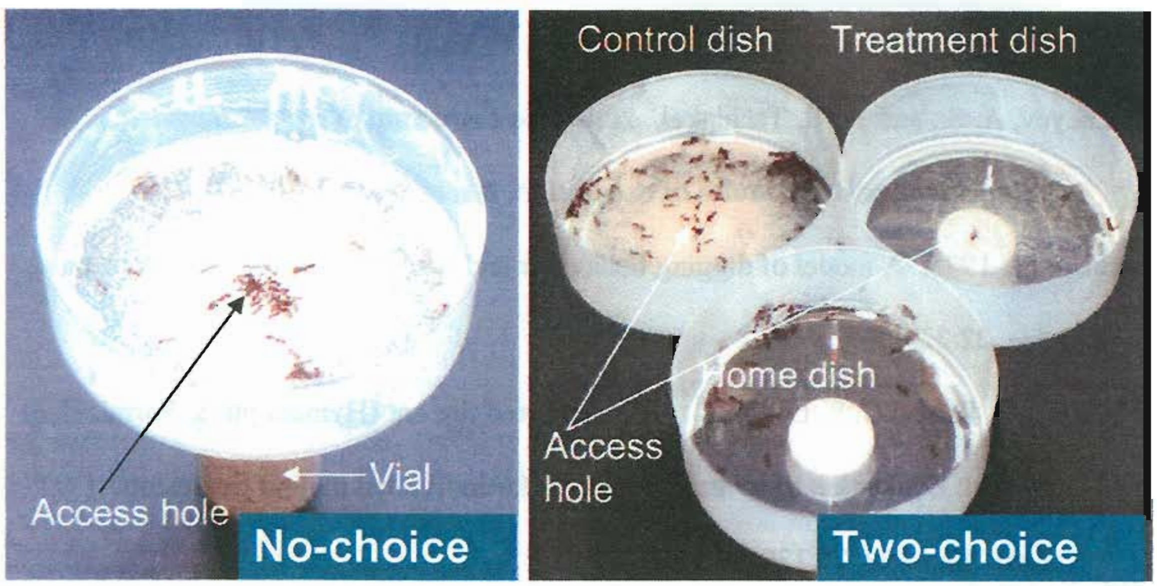


Figure 1. Apparatus used in bioassays. Workers dug the sand in the vials through access holes.

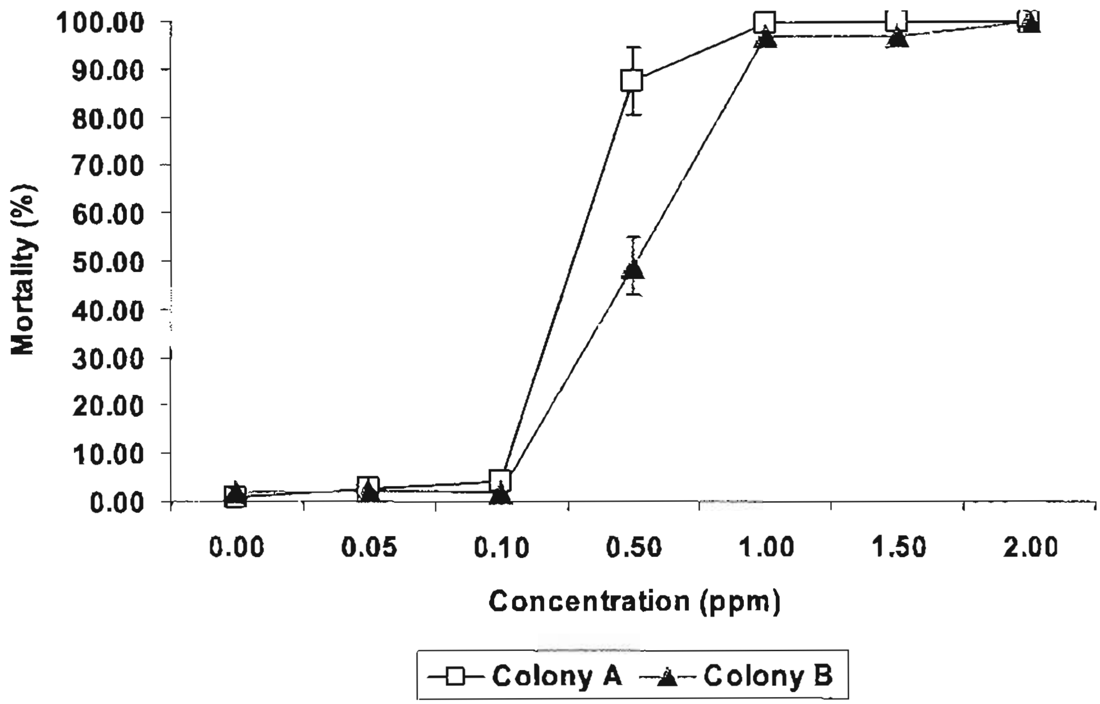


Figure 2. The 24 h mortality rate (mean ± SE) of fire ant workers in fipronil treated sand.

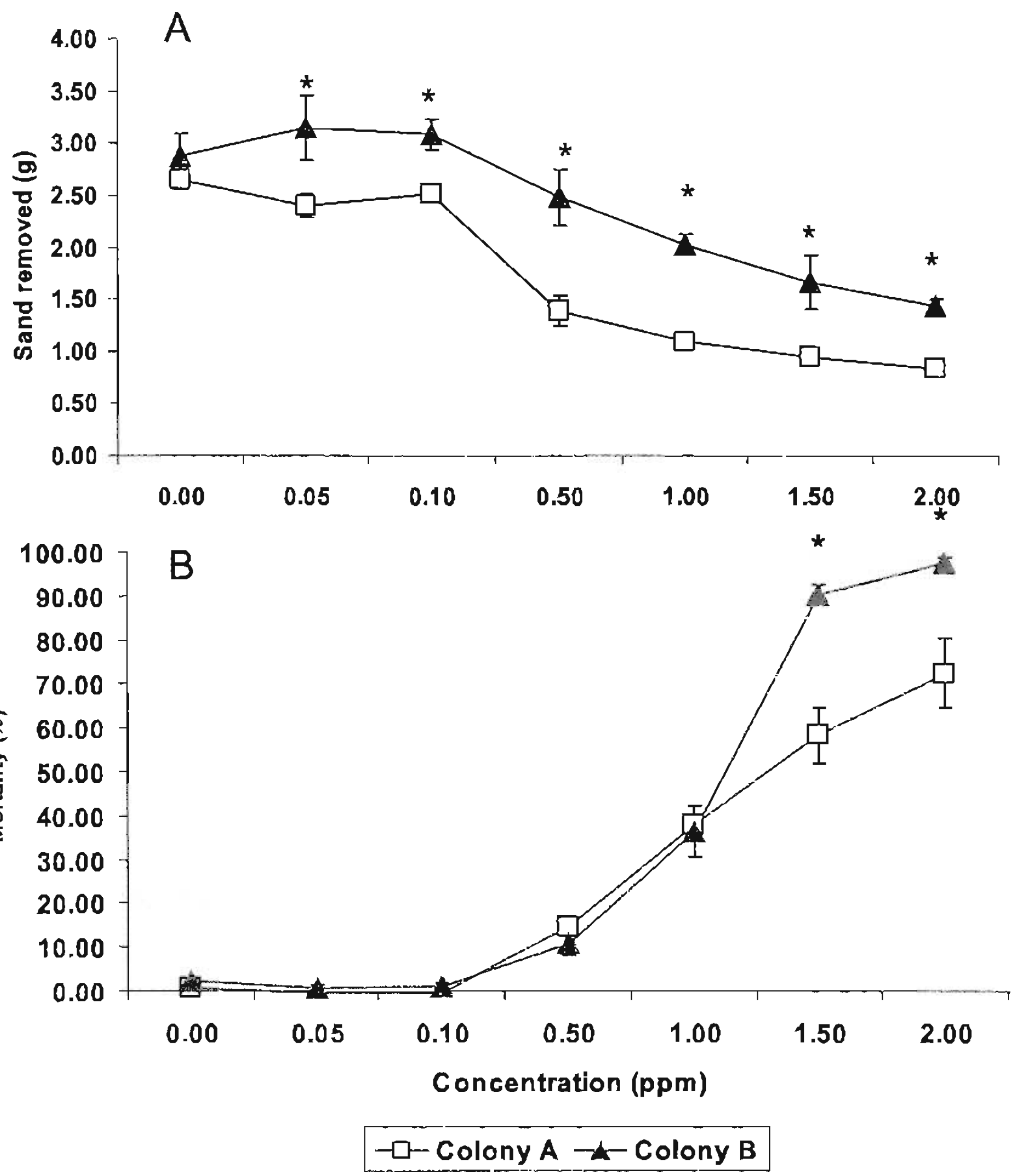


Figure 3. The amount of sand removed (A, mean ± SE) by fire ant colonies and their mortality rate (B, mean ± SE) in no-choice bioassays.

*: Colonies A and B are significantly different at $P < 0.05$.

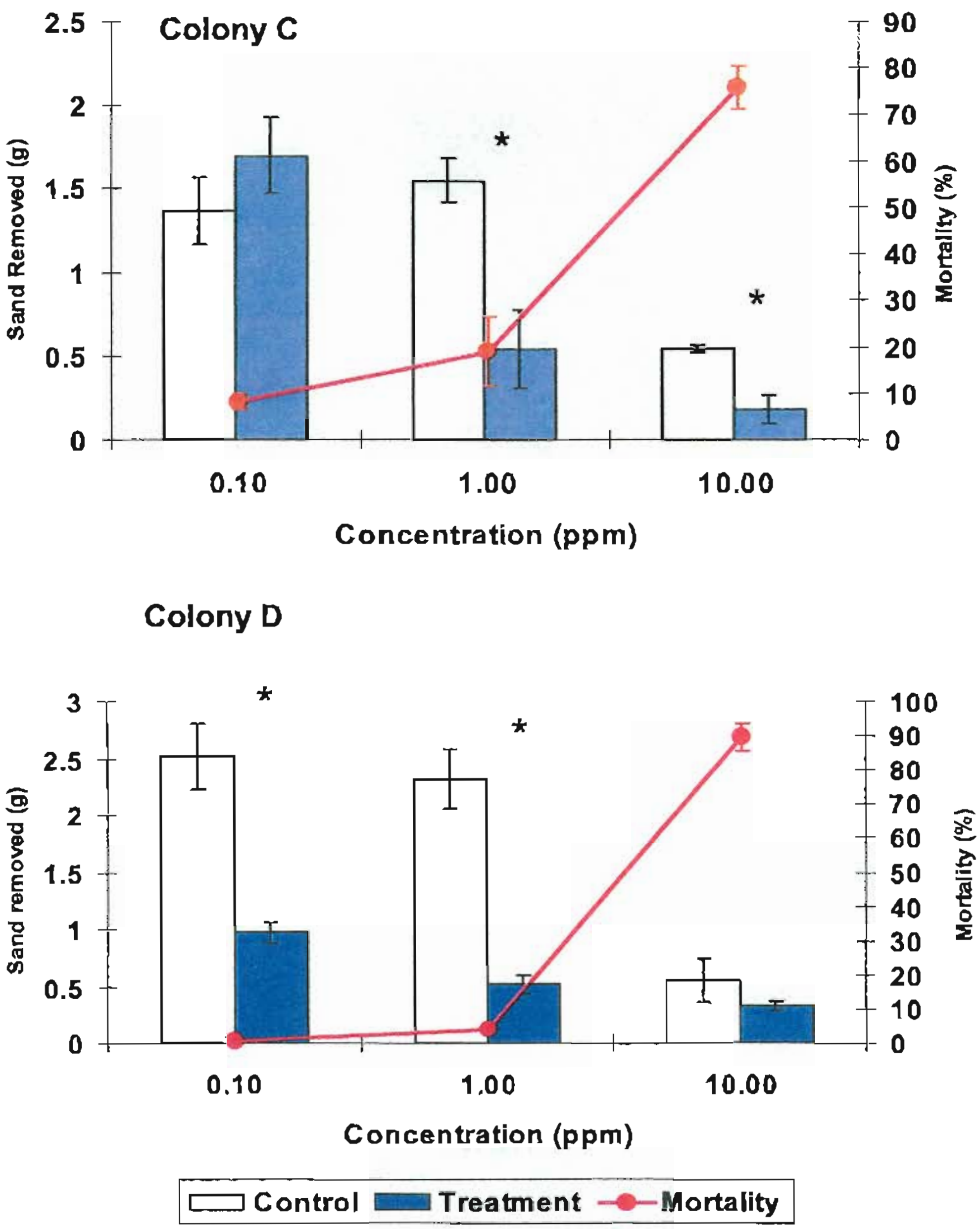
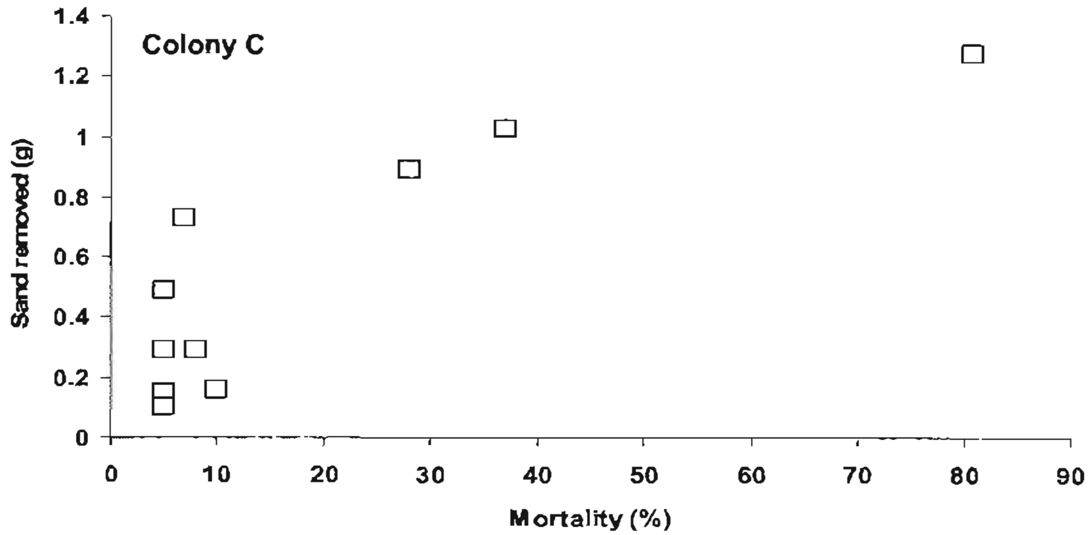


Figure 4. The amount of sand removed (mean ± SE) and mortality rate (mean ± SE) of fire ant workers from colonies C and D in two-choice bioassays.

*: Control and treatment are significantly different at $P < 0.05$.



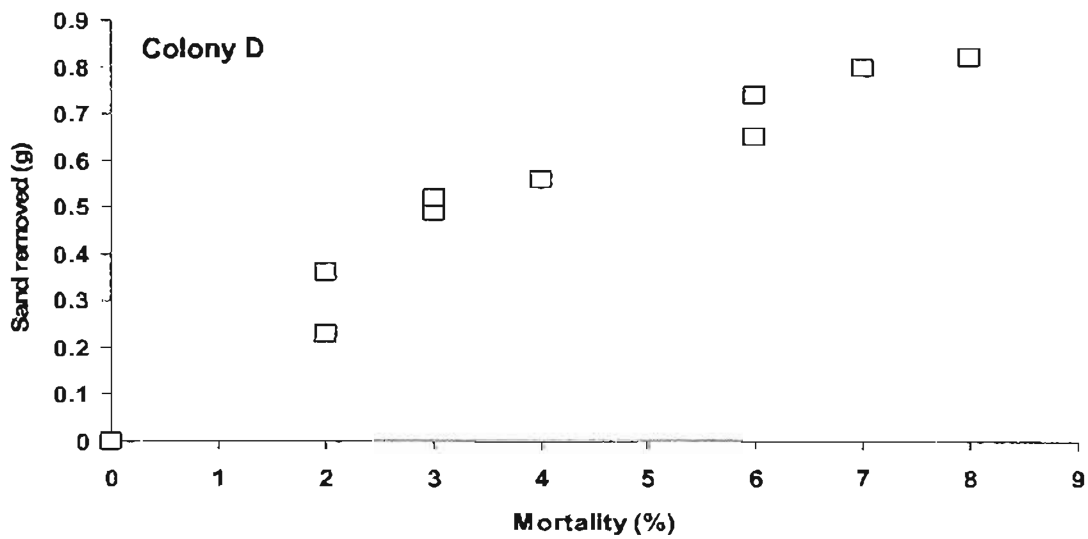


Figure 5. Correlations between amounts of sand removed from vials with 1.0 ppm fipronil treated sand by workers and their mortalities in two-choice bioassays. Correlation coefficient is 0.85 ($P = 0.002$) for colony C and 0.95 ($P < 0.0001$) for colony D.

AN IMPROVED METHOD FOR FAST AND EFFICIENT FIRE ANT COLONY SEPARATION

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SUMMARY

Substantial amount of brood was observed in the soil after using the standard water drip method for fire ant colony separation. A new procedure was developed to collect the brood that was not rescued by fire ant workers during the dripping process. Brood was collected by manually stirring the dirt submerged in the water, scooping out the floating brood, and using fire ant workers to separate brood from debris. The improvement involves: 1) increasing the water dripping rate to around 300 drops per minute for faster colony separation from the soil; 2) recovery of the un-rescued brood in the soil if so desired; and 3) rapid separation of brood from colony by the use of a repellant. This improved method resulted in significantly faster colony separation and significantly more brood recovery.

INTRODUCTION

The water drip method developed by Bank et al. (1981) is the commonly used procedure for separating fire ant colony from the soil. However, it was observed that at the recommended speed, the dripping process often had to run overnight and significant amount of brood was left in the soil without being rescued during the flooding process. A procedure was developed to collect the brood that was left behind in the soil, and a faster colony separation was obtained by increasing water dripping rate. Also, a method was developed for rapid separation of brood from colony by the use of a repellant.

SPEED UP COLONY SEPARATION FROM SOIL

Bank et al. (1981) recommended a water dripping rate of 20 to 40 drops per minute and at this rate it takes about 24 hours to flood out a colony from soil. For faster colony separation, the dripping rate was increased to around 300 drops per minute. The increased dripping rate still allowed mature ants to move upward to the soil surface while carrying the brood with them. The colony was separated from the soil in two to three hours at this increased dripping rate.

COLLECTION OF BROOD FROM SOIL AFTER WATER-DRIPPING PROCESS

After the ant mass was removed, the soil and water were manually stirred to allow the remaining brood to float on the water surface and then strained out. Worker ants were used to separate brood from debris. Healthy brood was moved to the ant nest while dead ones were placed in the refuse piles. Brood recovered in this way was 40 to 200% of that rescued by workers during the dripping process.

SEPARATION OF BROOD FROM COLONY USING FIRE ANT REPELLANT

Separation of brood from colony is often difficult. It was found that by using a suitable repellant, brood could be efficiently separated from workers. After ants with brood settled down in a Petri dish or "nest", a small cotton ball with a few drops of the repellent was placed at the center of the dish. The workers and reproductives were repelled out of the "nest" leaving the brood behind inside of the "nest". This process took only about 30 minutes. The chemical used is safe to brood, workers, and reproductives when used at an appropriate concentration.

CONCLUSIONS

Water dripping rate at approximately 300 drops per minute proved to be successful for faster colony separation from soil. The total amount of brood collected was substantially increased by manually stirring the dirt in the bucket after the ant mass was removed and then straining out the floating brood. The brood collected this way was greater than those rescued by worker ants. Fire ant workers proved to be efficient on separating brood from debris. Brood was efficiently separated from workers and reproductives by the use of a repellant.

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I thank Douglas A. Streett and M. Guadalupe Rojas, USDA-ARS, Stoneville, MS, for providing valuable comments on this manuscript, and Ling Xiao Zhang and Steve Kyei-Boahen, Delta Research and Extension Center, Stoneville, MS for critical reviews of the manuscript. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

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Areawide Suppression of Fire Ants: Demonstration Project in Mississippi, 2004

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INTRODUCTION

The USDA-ARS demonstration project for the suppression of imported fire ants has entered its fifth year. Participating states include: Florida, Mississippi, Oklahoma, South Carolina, and Texas. The areawide suppression project on imported fire ants in private pasture integrates biological control agents with the chemical bait products hydromethylnon and methoprene. An earlier report (Vogt et al., 2004) described the status of the demonstration project until early 2003 in Mississippi. The following is a report on the status of the USDA-ARS Areawide Suppression of Fire Ants Demonstration Project for 2004 in Mississippi.

MATERIALS AND METHODS

Mississippi's involvement in the project focused on protocol implementation against black/hybrid imported fire ants located in Grenada and Clay Counties (Fig. 1). Two sites located in Grenada County were selected for chemical bait treatment, and two sites located in Clay County were selected for the combined biological and chemical bait applications. Treatment areas consisted of at least 220 acres with a 280 acre minimum untreated contiguous border. Fifty circular plots (0.25acre) were established at each of our four sites: 20 plots in the treatment and 30 plots in the boundary. Treatment and boundary plots were monitored 4x and 2x/year respectively. Mound counts and hotdog bait attractants were conducted every sampling period with pitfall samples collected in the spring and fall only.

Multiple release attempts and yearly monitoring of the biological control agents *Thelohania solenopsae* (Microsporida) and the phorid fly, *Pseudacteon curvatus* began in 2002. In cooperation with USDA-APHIS, Gulfport, Mississippi, aerial applications of Extinguish® (A.I. Methoprene 0.75 lbs/acre)* and SiegePro® (A.I. hydromethylnon 0.75lbs/acre)* were applied yearly to the treatment areas of all four sites. A mound density threshold of 20% of the original spring counts was determined to be the trigger for reapplication.

RESULTS AND DISCUSSION

The trigger in Mississippi was set at 20% mound reinfestation. In the Fall of 2002, mound densities in both counties exceeded the trigger level (Fig. 2). The Mississippi Spring sampling was completed in early May, 2003 and aerial application of the chemical bait by USDA/APHIS and USDA/ARS personnel followed the 2003 Spring sampling (Fig. 2). At four weeks post- application the mound densities were below the trigger level in both counties. At twelve weeks post application, in mid-August the mound densities at the sites in Clay County had reached 23% of the Spring mound density. A 2004 spring bait application for Clay County (biocontrol) was delayed several times and cancelled due to weather. Grenada County did not require treatment because of a 2003 fall application. In fall 2004, mound densities at both counties exceeded the trigger level. Application of chemical bait was applied to both counties mid-October (Grenada on the 13th and Clay on the 18th) after several days of fog delays.

In addition to fire ants, eleven ant species were collected at the demonstration project treatment sites in Mississippi. Two of these species, *S. molesta* and *M. minimum* were found on bait samples located in plots with fire ants (Table 1).

Thelohania Summary

Since July 2002, three attempts have been made to introduce *Thelohania solenopsae* into two pasture sites in Clay County, Mississippi. In 2002, 82 mounds were each initially challenged with three gms of red imported fire ant brood inoculum. In April 2003, 186 mounds were provided *Thelohania*-infected brood following the project protocol. Follow-up sampling of the inoculated mounds in the fall of each year failed to yield active infected colonies.

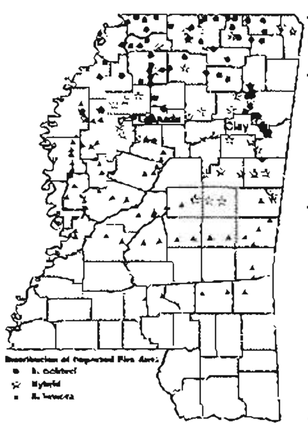
Members of the demonstration project in Florida traveled to Mississippi in April 2004 and administered infected brood to 275 nests between both sites at 5, 8, and 25* grams per nest (*only provided to 2 active mounds). In the fall of 2004, a single mound was recovered at Knox where the individuals were discovered to contain *Thelohania* spores. The mound was originally challenged with eight gm of infected brood in April 2004.

Phorid Fly Summary

The fire ant decapitating fly, *P. curvatus* was first released in two pastures in Clay Co., Mississippi during the Spring of 2002 and 2003. *P. curvatus* has become established on black and hybrid imported fire ants, *Solenopsis richteri* and *S. invicta* X *richteri*, respectively (Vogt and Streett, 2003). *P. curvatus*, as of September 2004, has dispersed to occupy an ellipsoidal area over 2249 km^2, or over 224,914 ha (Fig. 3). Average dispersal was 14.5 km/year with a range of 5.5 - 22 km/year. During mound sampling, time to first fly appearance ranged from about 10 seconds to 20 minutes.

Table 1. List of ant species captured at each of the areawide study sites. Names with asterisk (*) were only captured in pitfall traps.

Grenada County		Clay County	
Torrance	Woodland	Prima	Knox
Imported Fire Ant	Imported Fire Ant	Imported Fire Ant	Imported Fire Ant
Forelius pruinosus	*Forelius pruinosus*	*Forelius pruinosus*	*Monomorium minimum*
Monomorium minimum	*Monomorium minimum*	*Monomorium minimum*	*Solenopsis molesta*
Paratrichina vividula	*Solenopsis molesta*	*Solenopsis molesta*	*Forelius pruinosis**
Pheidole bicarinata	*Pheidole dentata**	*Paratrichina parvula**	*Paratrichina vividula**
Solenopsis molesta	*Hypoponera opacior**	*Paratrichina vividula**	
*Hypoponera opacior**	*Paratrichina vividula**	*Prenolepsis imperis**	
*Paratrichina longicornis**			
*Paratrichina parvula**			
*Pheidole dentata**			
*Tapinoma sessile**			



Site	Identity	Number	Percent
Clay County			
Knox			
	BIFA	42	86%
	Hybrid	7	14%
Prima			
	BIFA	25	57%
	Hybrid	19	43%
Grenada County			
Woodland			
	BIFA	4	8%
	Hybrid	43	90%
	RIFA	1	2%
Torrance			
	BIFA	11	24%
	Hybrid	34	74%
	RIFA	1	2%

Fig. 1. Delineation of imported fire ant species composition throughout Mississippi with focused attention on participating areawide counties. Species were identified via cuticular hydrocarbon and venom alkaloid analyses by Robert Vander Meer USDA-ARS.

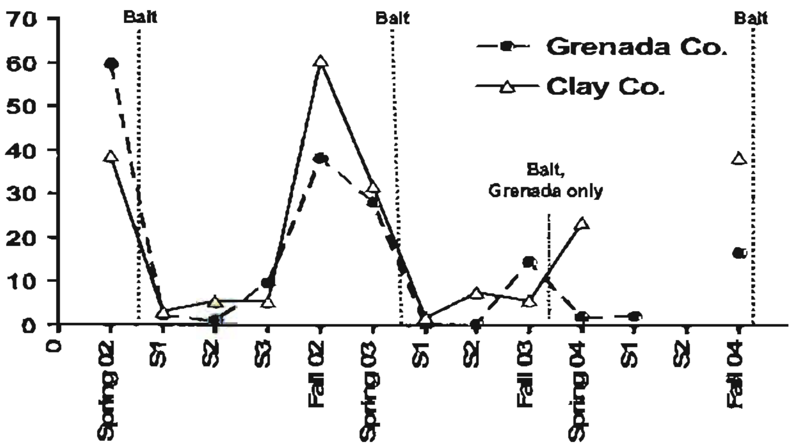


Fig. 2. Average number of mounds assessed per chemical-bait treated acre over time. S1, S2, and S3 represent summer sampling events. Weather and lack of personnel prevented sampling during the summer of 2004.

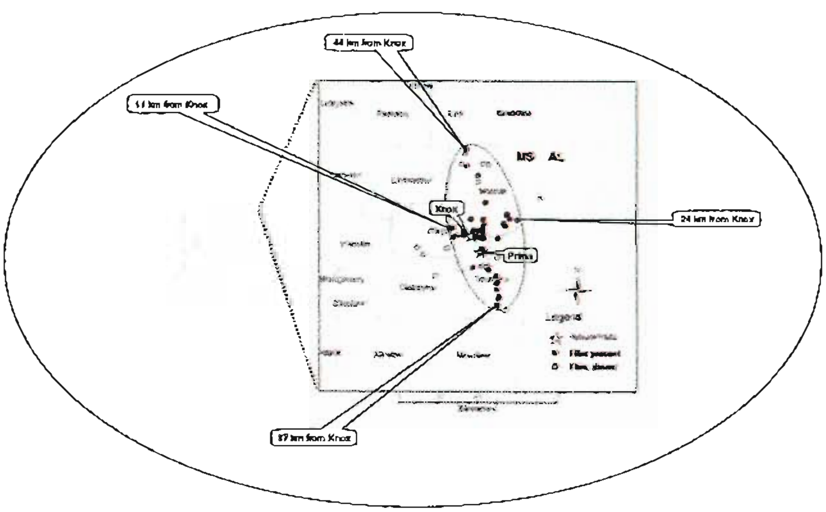


Fig. 3. Dispersal pattern of *P. curvatus* from Knox and Prima release sites in Clay County, Mississippi.

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*Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

SURVEY OF BACTERIA AND FUNGI ASSOCIATED WITH RED IMPORTED FIRE ANTS *Solenopsis invicta* BUREN: AN EFFORT TO DISCOVER POTENTIAL MICROBIAL CONTROL AGENTS

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INTRODUCTION

Background

Red imported fire ant (RIFA, *Solenopsis invicta* Buren) is one of the major pests throughout southeastern of the United States. This aggressive pest is difficult to control and has caused problems in agricultural settings as well as human and animals. Area-wide control efforts are currently being conducted in the midsouth area; the efforts include utilizing biological control agents to control RIFA. For example, phorid flies *Pseudacteon tricuspis* and *P. curvatus* as well as a microsporidian *Thelohania solanapsae* have been tested and released in many areas. A report (http://http.fireant.ifas.ufl.edu) indicated that potential annual benefits of using natural enemies to control fire ants are $4.6 billion (agriculture: $499 million, households: $2.7 billion, government, schools and institutions: $203 million, and other business: $1.1 billion).

Research objective

As a part of the control efforts, in Mississippi we are conducting a study to determine the bacteria and fungi present in RIFA mounds. Once identified, many of the isolates will be tested for their potential as biological control agents.

MATERIALS AND METHODS

1. Field Sampling

Sampling dates and locations:

Collection time	March, July, and November 2004
Locations	Leake, Madison, and Hinds Counties in Natchez Trace Parkway, MS **(Figure 1)**

Five active mounds (2000 ml of volume per mound per collection time per location) containing RIFA, soil mounds and plant debris were collected and transported to the laboratory.

Each sample was processed using microbiological standard procedures. For isolation of bacteria, samples were plated on trypticase soy agar (TSA) amended with 50 mg/L Nystatin (antifungal agent). For isolation of fungi, samples were plated on Sabouraud's dextrose agar containing 2% yeast extract (SDAY) and amended with 300 mg/L streptomycin sulfate and 100 mg/L chlortetracycline (antibiotics). Colonies obtained were selected based on their morphological characteristics, subcultured to obtain pure culture, and stored at room temperature and -80°C.

2. Identification of Bacteria & Fungi

Bacteria were characterized using fatty acid methyl esters (FAMEs) analysis technique. Each bacterial isolate was streaked onto trypticase soy broth agar (TSBA) and incubated for 24-48 h at 28±1°C. A summary of FAMEs analysis procedures is outlined in **Figure 2.**

Fungi will be identified based on conidiogenesis according to standard mycological references or from sexual reproduction when it occurs.

To confirm the identification, representative isolates of bacteria and fungi will be characterized using molecular techniques.

3. Evaluation of Bacteria and/or Fungi as Potential Microbial Control Agents – Preliminary Study (Figure 3)

Sixty colonies were established in the Rearing Center, Department of Entomology and Plant Pathology, Mississippi State University. These colonies (fire ants and soil mounds) were placed in Sterilite® plastic boxes (34.29 x 20.32 x 10.16 cm).

Individual isolates of identified bacteria and fungi were grown on TSA and SDAY, respectively. Two crickets were weighed and placed in the center of each bacterial or fungal plate. Each plate was placed in the middle of colony box. TSA and SDAY plates without a bacterium or a fungus, respectively, plus two crickets were used as controls. The numbers of ants foraging on crickets 1 h after crickets were added (measured by rate: Rate 0 = 0 ant, rate 1 = 1-10, rate 2 = 11-25, rate 3 = 26-50, rate 4 = 50-100, rate 5 = 101-200, and rate 6 >200 ants) were recorded. Crickets were added daily up to 4 days and any type of behavioral changes was also recorded daily. At the end of the experiment, the remaining crickets were weighed.

RESULTS & DISCUSSIONS

More than 4500 bacterial and fungal isolates were collected. Approximately 1170 bacterial isolates have been analyzed using FAMEs technique and eighty one species were identified. Since we deal with many isolates to identify, FAMEs method was selected to initially identify bacteria. This technique was selected because it offers cost effective, efficient, rapid, sensitive methods to characterize and identify microorganisms, including bacteria. In comparison, the traditional identification methods are laborious and time consuming. The most common bacteria identified (31 species) are listed in Table 1 below where *Bacillus cereus* subgroup A and B appeared to be the most common species recovered. Fungi have been tentatively grouped and they consisted of more than 200 groups. These fungi will be further identified using morphological features.

Table 1. Mean percentage of bacterial species isolated from red imported fire ant, plant debris and soil mounds collected from Natchez Trace Parkway in Mississippi across three sampling dates in 2004*

Species	HINDS	LEAKE	MADISON
Bacillus cereus subgroup A	3.25	1.80	5.40
Bacillus cereus subgroup B	0.50	0.60	0.15
Bacillus circulans	0.15	0.15	0
Bacillus coagulans	0.05	0.05	0.05
Bacillus lentimorbus	0.25	0.55	0
Bacillus megaterium subgroup A	0.25	0.25	0.15
Bacillus megaterium subgroup B	0.10	0.05	0
Bacillus mycoides subgroup A	0.75	0.35	0.25
Bacillus mycoides subgroup B	0.60	0.55	0.75
Bacillus pumilus subgroup B	0.60	0	0.15
Bacillus sphaericus subgroup III	0.40	0.10	0
Bacillus sphaericus subgroup IV	0.35	0.10	0.15
Bacillus spahericus subgroup V	0.30	0.80	0.15
Brevibacillus laterosporus	0.05	0.10	0.05
Cedecea davisae	0.15	0	0
Klebsiella pneumoniae ozaenae	0.10	0.05	0.05
Micrococcus lylae	0.10	0.05	0.05
Nocardia globerula	0.05	0.20	0
Paenibacillus alvei	0	0.25	0
Paenibacillus apiarus	0.05	0.15	0
Paenibacillus gordonae	0.20	0	0.05
Paenibacillus macerans subgroup B	0.10	0.20	0.30
Paenibacillus polymyxa	0.45	0.60	0.05
Pseudomonas chlororaphis	0.40	1.05	0.10
Pseudomonas putida biotype A	0.10	0.80	0.10
Rhodococcus equi subgroup B	0.10	0.25	0.10
Salmonella typhimurium subgroup B	0.10	0.05	0
Stenotrophomonas maltophilia	0.05	0.10	0
Yersinia pseudotuberculosis	0	0.15	0
Bacillus marinus	0.10	0.05	0
Micrococcus luteus subgroup B	0.10	0	0.05
Others**	1.85	1.00	0.35
Unknowns***	3.25	8.50	2.05

* The three sampling dates were March, July, and November 2004.

** Others consisted of 50 bacterial species with mean percentage < 0.10%.

*** Unknowns represent bacterial isolates that were unidentified.

Many of these species, particularly the ones that were shown to be pathogens for other insects, will be evaluated for the biological control potential (similar to preliminary study described in "materials and methods"). For the laboratory scale experiment, artificial diet will be used to limit the influence of other microorganisms except the one tested.

ONGOING AND FUTURE STUDIES

1. To complete FAMEs analysis for the remaining bacterial isolates.
2. To identify fungi using standard mycological references.
3. To confirm identification of representative isolates of bacteria and fungi using molecular techniques.
4. To calculate taxa diversity indexes and conduct statistical analysis (Randomized Complete Block Design with a log10-transformed applied where appropriate).
5. To test selected bacterial and fungal isolates as potential microbial control agents against RIFA in laboratory setting and cage experiment.

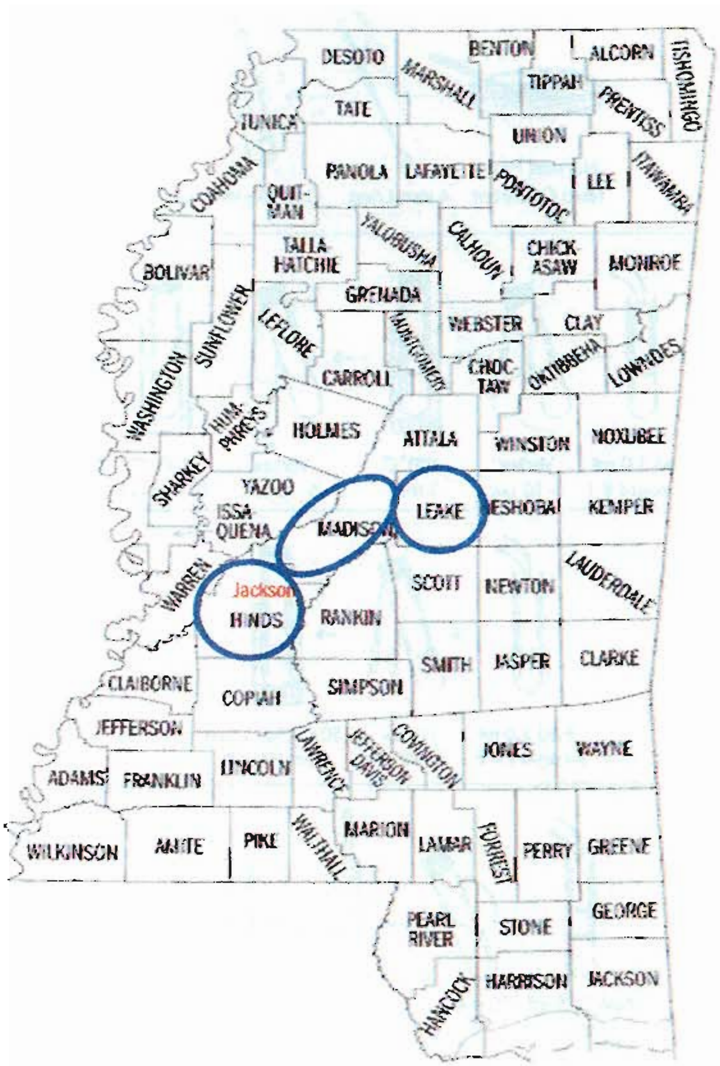

Figure 1.
Map of counties in Mississippi. Circled in blue are the three counties (Leake, Madison, and Hinds) where samples from RIFA mounds were collected.

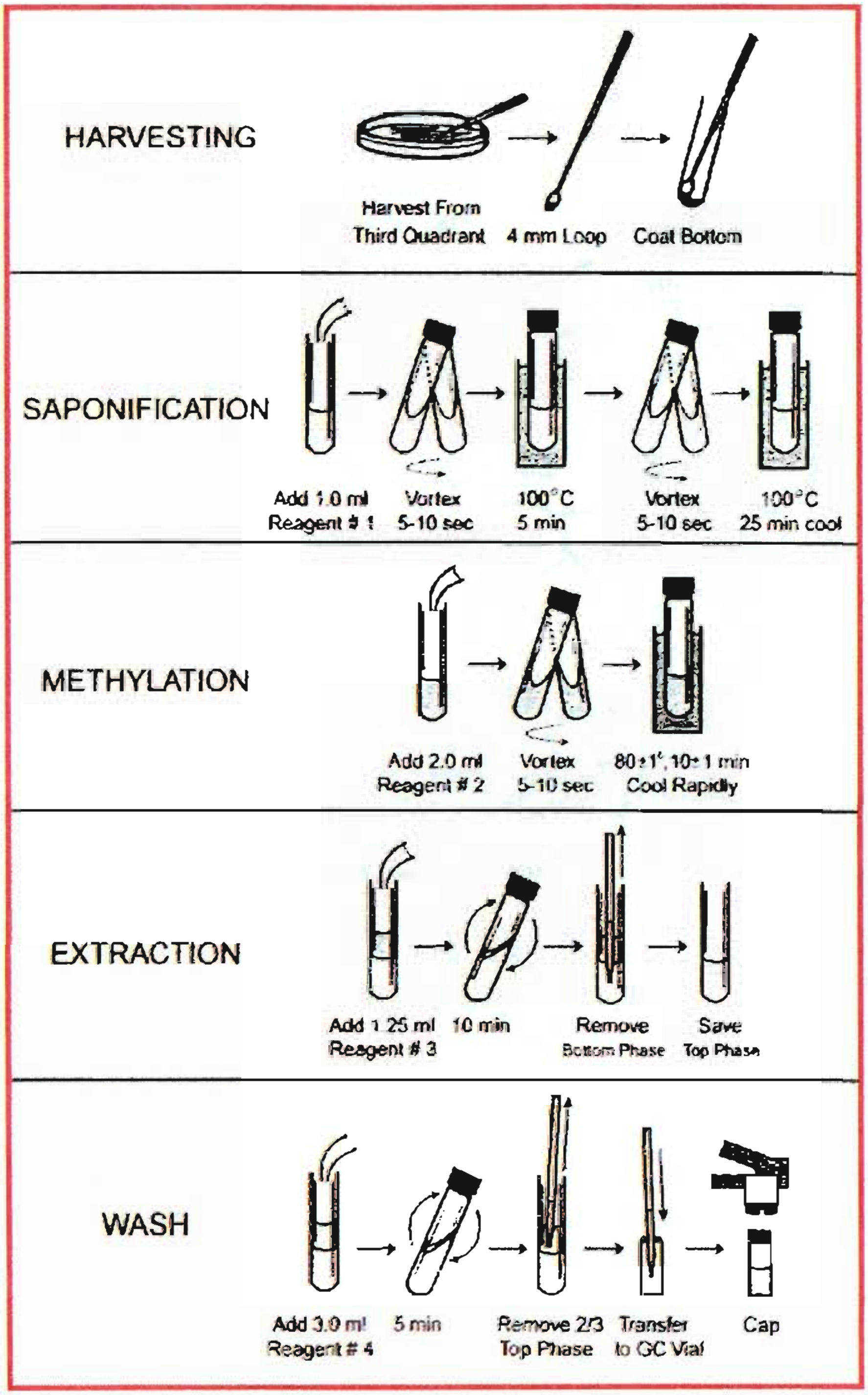

Figure 2. Diagram of FAMEs analysis procedure

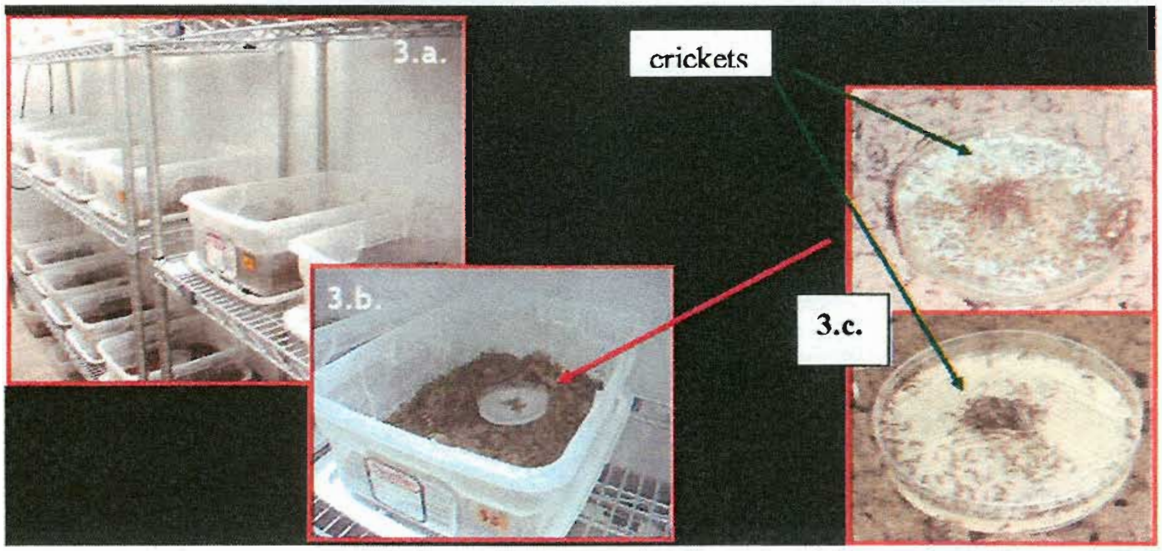

Figure 3.
a) Fire ant colonies were established in boxes for potential microbial control (preliminary) study, b) Bacterial or fungal plate with crickets was placed in the middle of ant colonies, c) Close-up images of bacterial and fungal plates with crickets; note the fire ants that foraged the crickets.

Toward Fire Ant Biological Control I Isolation and Molecular Characterization of Bacteria from the Red Imported Fire Ant (*Solenopsis invicta*) Midgut

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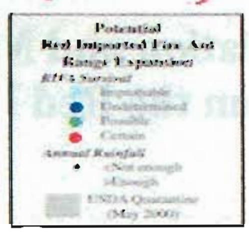
Abstract

Bacteria were isolated and cultured from the red imported fire ant (*Solenopsis invicta*) midgut. The small-subunit ribosomal RNA gene, (16s rRNA gene, approximately 1,500bp) was amplified from bacterial genomic DNA using the Polymerase Chain Reaction (PCR) and consensus sequence primers. Restriction Fragment Length Polymorphism (RFLP) analysis revealed 10 unique profiles, indicating that at least 10 different bacteria are present in the red imported fire ant midgut. The 16s rRNA gene sequence was determined and queried against the NCBI genetic database. The results identified all isolates to at least the genus level. Biochemical characterizations were also performed on these isolates. In addition, using a species-specific PCR-based identification method, the distribution of these 10 midgut bacteria in fire ant colonies from different sources was investigated.

Introduction

- **Red imported fire ant,** *Soleonpsis invicta*, Buren (Hymenoptera: Formicidae) was accidentally introduced into the U.S.A. through the port of Mobile, Alabama in the 1930s, and causes billions of dollars of losses each year.
- Bacteria play a key role in the biological control of insect pests. The long-term goal of our research is to use genetically modified bacteria as an alternative strategy for fire ant control.
- We describe the isolation and characterization of bacteria from the midgut of 4th instar fire ant worker larvae. Using PCR-RFLP and sequence analysis of the bacterial 16s rRNA gene, we identified 10 bacterial isolates to at least the genus level. We also investigated the colony distribution of these isolates.

Potential United States range expansion of the invasive fire ant, *Solenopsis invicta*

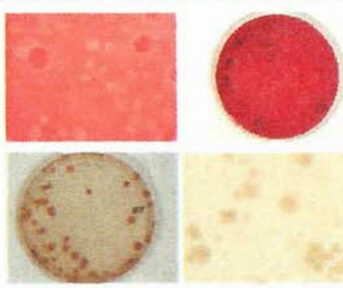


Materials and Methods

- Fire ant larvae, (4th instar) surface sterilization
- Midgut sac dissection, homogenization
- Spread on Blood Agar plates, incubation
- Representative colony selection.
- Genomic DNA extraction
- Bacterial 16s rRNA gene amplification and RFLP analysis
- Biochemical characterization of bacterial isolates
- Antimicrobial susceptibility test
- Isolate identification by 16s rRNA gene sequencing
- PCR screening to determine distribution

Results

- **Phenotypic characterization of isolates**



- Most colonies grew up to 4 mm in diameter after 24 hours incubation at 37°C, some colonies were only 1/3 the diameter of the majority of colonies.
- β and γ - hemolysis was observed.
- According to growth characteristics and colony morphology, 36 well-isolated colonies were selected for further characterization and identification.

Figure 1. Phenotype of the isolated bacteria on Blood Agar plates supplemented with 5% sheep blood and Nutrient agar plates.

- **PCR amplification of the 16s rRNA gene and RFLP analysis**

Bacterial genomic DNA was extracted and the 16s rRNA gene was amplified using universal primers. An approximately 1.5 kb fragment was produced (nearly full length).

All amplified 16s rRNA products were digested with *Hae*III, *Rsa*I and separated on a 2% agarose gel. RFLP analysis resulted in 10 different restriction patterns for the amplified 16s rRNA gene products.

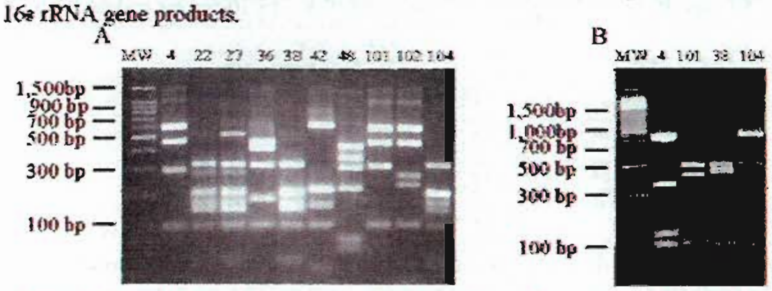


Figure 2. PCR- RFLP profile of 16s rRNA genes from the midgut bacteria of the red imported fire ant obtained by *Hae*III and *Rsa*I digestion.
(A) *Hae*III restriction digestion of amplicons from the 16s rRNA genes of 10 selected isolates.
(B) *Rsa*I restriction digestion of 16s rRNA amplicons from isolates 4, 101, 38 & 104.

- **Biochemical characterization of bacterial isolates selected by 16s rRNA PCR-RFLP**

The 10 selected isolates were identified according to Gram reaction, Oxidase activity, MacConkey Agar Plates, API-M medium, API-OF medium.

The biochemical properties of the isolates were determined using the Analytical Profile Indexes API- 20E kit, shown in Table 1 and Figure 3.

Table 1. Biochemical characterization of 10 bacterial isolates from red imported fire ant midguts

Reaction/Enzymes	4	22	27	36	38	42	48	101	102	104
Motility	+		-	-	+	+	-	+	+	-
MacConkey	-	+	+	-	+	+	+		-	+
Cytochrome-Oxidase	+	-	-	+	+	+	+	+	-	-
Arginine dihydrolase	+	+	-	+	+	+	-	+	-	+
Citrate utilization	-	+	-	-	+	+	+	+	+	+
H_2S production	-	+	-	-	+	-	+	+	+	+
Urease hydrolysis	-	-	-	+	+	+	+		-	-
Indole production	+		+	-	-	-	-	-	-	-
Acetoin production	-	+	+	+	+	+	-	-	+	-
Gelatinase hydrolysis	-	-	-	-	+	+	+	+	-	+
Glucose fermentation/oxidation	+	+	+	+	+	+	-	+	+	+
Mannitol fermentation/oxidation	-	+	+	-	-	-	-	-	-	+
Sorbitol fermentation/oxidation	+	+		-	+	-	-	+	-	+
Arabinose fermentation/oxidation	-	+	+	+	+	+	+	+	-	-

Bacteria #104: Profile code: 4-3-0-6-7-2-1 **Result: *Serratia***

Figure 3. Biochemical characterization of bacterial isolate 104 from red imported fire ant midguts

- **Antimicrobial susceptibility test**

The antimicrobial susceptibilities of the 10 selected isolates were determined using the disk diffusion test procedure (Kirby-Bauer).

Isolates were considered as resistant (R), intermediate (I) and susceptible (S) following the disk diffusion zone diameter chart.

Table 2. Antibiotic resistance of 10 bacterial isolates from red imported fire ant midguts

	4	22	27	35	38	42	48	101	102	104
Ampicillin	S	R	I	S	S	R	R	S	S	R
Chloramphenicol	S	S	S	S	S	I	R	S	S	S
Doxycycline	S	S	R	R	S	R	R	S	R	R
Erythromycin	R	R	I	S	I	R	I	I	S	R
Gentamicin	S	S	S	S	S	S	R	S	S	S
Kanamycin	R	S	S	S	S	R	R	S	S	S
Nalidixic Acid	R	S	S	R	S	R	R	I	R	S
Neomycin	S	S	S	S	S	I	R	S	S	S
Spectinomycin	S	S	S	S	R	S	R	S	I	S
Streptomycin	S	S	S	S	S	I	R	S	S	S
Tetracycline	S	S	R	R	S	R	R	S	R	R
Zeocin	R	S	S	R	S	R	R	S	R	R

- **Isolate identification by 16s rRNA gene sequencing**
  - Gel purified PCR products of the 16s rRNA gene fragments from the 10 isolates were cloned into the TA cloning vector, Topo2.1.
  - 16s rRNA gene sequence of each isolate was compared with known 16s rRNA gene sequences in the Gen Bank database using the BLAST search algorithm.

Table 3. NCBI BLAST results for the 16s rRNA gene sequence from the 10 fire ant midgut bacterial isolates

Isolate #	Identification	GenBank Accession #	NCBI Blasting Accession #	E-Value	Similarity (%)
4	*Enterococcus sp.*	AY946232	AY321376	0	99
22	*Enterobacter sp.*	AY946233	Z96078	0	99
27	*Kluyvera cryocrescens*	AY946234	AF310218	0	99
36	*Lactococcus garvieae*	AY946235	AY699289	0	99
38	*Uncultured bacterium*	AY946236	AJ487029	0	100
42	*Pseudomonas aeruginosa*	AY946237	AB126582	0	99
48	*Achromobacter xylosoxidans*	AY946238	AF411021	0	99
101	*Bacillus pumilus*	AY946239	AB020208	0	99
102	*Listeria sp.*	AY946290	AL596173	0	95
104	*Serratia marcescens*	AY946291	AB061685	0	99

- **PCR screening to determine the distribution of the midgut bacteria among different colonies**

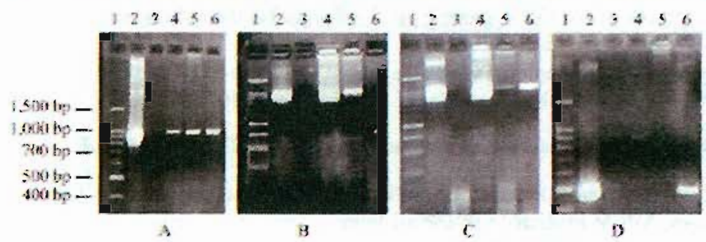


Figure 3. Bacterial species-specific PCR screening of the bacteria in red imported fire ant worker midguts from lab-maintained colonies and a field colony,
(A), (B), (C), (D) Bacterial species-specific PCR screening for isolates 27, 36, 42 and 48, respectively.

Discussion

- 10 bacterial species were isolated from 4th instar larval midguts of red imported fire ant workers. By using PCR-RFLP analysis of the 16s ribosomal RNA gene and DNA sequencing of this gene, we were able to group and identify these isolated bacteria to at least the genus level.
- The species-specific PCR assay was based on our original 16s rRNA sequence of the 10 isolated bacteria Preliminary investigations of specificity indicate that these primer sets can be used on fire ant colonies from different sources and the expected amplification fragments can be obtained using the specific primer pairs.
- This screening is a preliminary study on the distribution of these bacteria in the fire ant colonies. In order to ascertain the distribution of these 10 isolated bacteria in fire ant colonies from different sources, a more comprehensive analysis is necessary and a study involving fire ant colonies collected from more than 30 locations across the USA is in progress.

Future Directions

For the purpose of insect pest control using these bacteria, they must be transformed with exogenous DNA. Three of the bacteria from the fire ant midgut can be successfully engineered by introducing a plasmid which encodes a red fluorescent protein (DsRed) and successfully re-introduced into the fire ant larvae. The bacteria studied here will be investigated for their use as shuttle vehicles for the introduction of foreign genes and the expression of foreign proteins in the fire ant, allowing the bacteria to be used as an alternative tool for the biological control of fire ants.

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Acknowledgments We would like to give special thanks to the staff of Dr. Vinson's lab for maintaining the fire ant colonies used for the study. This project was supported by a grant from the Texas Imported Fire Ant Research and Management Project.

Toward Fire Ant Biological Control II Genetic Engineering of Midgut Bacteria From The Red Imported Fire Ant (*Solenopsis invicta*)

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Abstract

In our previous study we isolated 10 bacterial species from 4th instar larval midguts of the red imported fire ant (*Solenopsis invicta*). Three isolated strains (*Kluyvera cryocrescens*, *Serratia marcescens* and isolate #38) can be genetically modified with the plasmid vector, pZeoDsRed. High expression levels of DsRed in these bacteria were observed and the plasmid can be stably maintained in these gut bacteria at 37°C in the absence of antibiotic selection. The transformed gut bacteria were successfully re-introduced into fire ant colonies and survived in the fire ant gut for at least 7 days. Strong fluorescence of DsRed was detected throughout the larval stage. Upon pupal emergence, 7 days after re-introduction, transformed bacteria can still be rescued, however, most were passed out in the meconium. We further demonstrated that the engineered bacteria could be spread within the colony by feeding this meconium to naive larvae with the aid of worker fire ants.

Introduction

- The Red Imported Fire Ant, *Solenopsis invicta* Buren, (*Hymenoptera: Formicidae*), is one of the most important agricultural and urban pests in the United States, the tremendous impact on rural and urban environments makes the total annual cost to be estimated to several billion dollars.

Eggs, 3rd and 4th instar larvae, pre-pupa, pupa and adult fire ant worker

- The life cycle consists of four main stages: egg, larvae, pupae and adult.
- To evaluate the potential use of bacteria from the midgut of fire ants in control applications, three of the bacteria strains can be genetically modified and transformed with a shuttle vector expressing a DsRed protein and a bleomycin resistance gene.
- The shuttle vector is stably maintained and expressed in these three bacteria and re-introduced to fire ant colonies. Maintenance was observed in the colony 7 days after re-introduction. Furthermore, the genetically modified bacteria can be transmitted from transformed-bacteria-fed individuals to naive larvae within the colony, which were not directly fed transformed bacteria.

Materials and Methods

- ✓ Bacterial strains, recombinant plasmid and red imported fire ant colonies
- ✓ Competent cell preparation and bacterial transformation
- ✓ Plasmid detection of pZeoDsRed in transformed bacterial strains
- ✓ Plasmid stability test
- ✓ Re-introduction and detection of transformed bacteria in fire ant larvae

Result

- **Introduction and expression of the DsRed gene in fire ant midgut bacteria**

✓ pZeoDsRed plasmid was introduced into *K. cryocrescens*, *S. marcescens* and isolate #38 strains by electroporation.

✓ Both ble^r and DsRed genes were expressed in all 3 transformed strains. All Zeocin resistant colonies were DsRed positive and showed strong fluorescence under appropriate illumination.

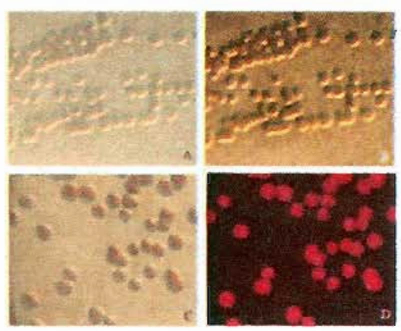


Figure 1. DsRed fluorescence in wild type and pZeoDsRed transformed bacterial isolate #38. Phenotypes of wild type strain under natural light (A) and the Rhodamine filter (B). Phenotypes of the isolate #38/ pZeoDsRed strain under natural light (C) and the Rhodamine filter (D).

- **PCR amplification of the 16s rRNA gene and RFLP analysis**

✓ The transformed bacterial strain contains an additional fragment that co-migrated with intact pZeoDsRed plasmid, this additional fragment was not found in the control bacteria.
✓ In Southern blot DNA hybridization, the size of the hybridizing band is 3.8 kb, which is the expected size of the linearized plasmid DNA, indicating that the pZeoDsRed plasmid was in transformed bacteria. The probe failed to hybridized with plasmid DNA extracted from wild type non-transformed bacteria.

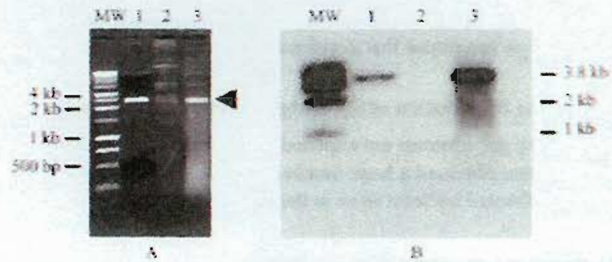


Figure 2. pZeoDsRed Plasmid DNA recovered from the transformed bacterial strain, *K. cryocrescens*/ pZeoDsRed.
(A). Agarose gel electrophoresis of non-digested plasmid DNA isolated from *K. cryocrescens* and the *E. coli* Top 10 strain.
(B). Southern blot of *Eco*RI digested plasmid DNA from the transformed *K. cryocrescens*/ pZeoDsRed strain. The blot was hybridized with a radio-labeled DsRed gene.

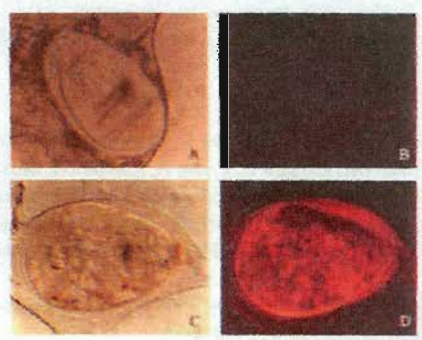
- **Plasmid stability test**

✓ The stability of the pZeoDsRed plasmid maintained in the fire ant midgut bacteria was assayed at both 37°C and 22-24°C, without Zeocin™ selection pressure.
✓ The pZeoDsRed plasmid can be stably maintained in the 3 host strains without loss of expression at 37°C.
✓ pZeoDsRed was stable (100% stability) on LB plates without Zeocin selective pressure at room temperature in isolate #38.
✓ At room temperature, the pZeoDsRed expression level in *K. cryocrescens* was too low to count efficiently, however, the plasmid was still maintained in the transformed bacteria as confirmed by using an additional test: higher expression levels can be obtained by re-culturing the plate at 37°C or re-streaking the colonies on a Zeo[R] plate.
✓ Expression of pZeoDsRed in the *S. marcescens* strain was low at room temperature without antibiotic selection, with most of the colonies being non-fluorescent after 9 days re-streaking.

- **Re-introduction of transformed midgut bacteria into the fire ant colony**

✓ Strains of *K. cryocrescens*, *S. marcescens* and isolate #38 carrying the pZeoDsRed plasmid were successfully re-introduced to the fire ant colony by individually feeding the larvae.
✓ The transformed bacteria survived and can be stably maintained in 4th instar larvae and the heterologous protein DsRed is highly expressed in the midgut of fire ant larvae 72 hrs after feeding.

Figure 3. DsRed expression in the midgut of 4th instar larvae from the red imported fire ant. Midgut of 4th instar larvae fed wild type bacteria, *K. cryocrescens*, and images were captured under natural light (A) and with an attached Rhodamine fluorescence filter (B). Midgut of 4th instar larvae fed by transformed bacteria *K. cryocrescens* / pZeoDsRed strain, natural light (C) and the Rhodamine fluorescence filter (D).



- **Detection of the pZeoDsRed in newly emerged pupae**

✓ Upon pupation and meconium expulsion (7 days post-bacteria feeding), most of the transformed bacteria were secreted and only minimal red fluorescence was detectable under the microscope in the fire ant. .

✓ DsRed fluorescent positive bacteria were successfully rescued from pupae that developed from *K. cryocrescens*/ pZeoDsRed fed larvae.

✓ No colonies with DsRed fluorescence positive clones were rescued from the PBS solution (used in the last step of surface sterilization and also plated on the same medium), indicating that the DsRed fluorescence positive clones rescued were not from surface contamination.

✓ No DsRed fluorescent positive clones were rescued from pupae that developed from isolate #38/ pZeoDsRed and *S. marcescens*/ pZeoDsRed fed larvae, however, approximately 500 DsRed fluorescent positive colonies were rescued from pre-pupae that developed from *S. marcescens*/ pZeoDsRed fed larvae.

- **PCR screening to determine the distribution of the midgut bacteria among different colonies**

✓ During the pre-pupal stage, the gut contents are expelled in the form of meconium.

✓ Bacterial rescue from meconium obtained a large number of strong red fluorescence bacterial colonies, confirming that most of the transformed bacteria were in the meconium.

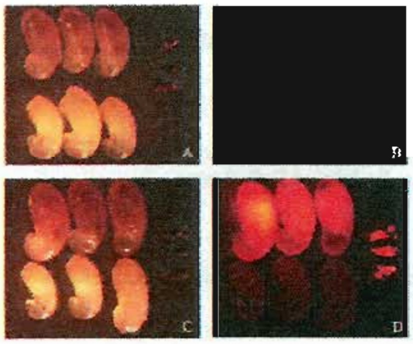


Figure 4. Bacteria passed out through meconium shed following pupal formation. 4th instar larvae (upper left), pre- pupae (lower left) and the shed meconium (right) are shown in each sub-figure. Phenotypes of meconium, 4th instar larvae, pre- pupae derived from wild type *K. cryocrescens* strain feeding, under natural light (A) and with an attached Rhodamine fluorescence filter (B). DsRed expression in meconium, 4th instar larvae, and pre-pupae following *K. cryocrescens* / pZeoDsRed strain feeding, (C) and (D).

Discussion

✓ Three fire ant midgut bacteria, *K. cryocrescens*, *S. marcescens* and isolate #38, were successfully transformed with a DsRed-encoding shuttle vector. The transformation efficiencies and DsRed fluorescence levels were significantly different.

✓ The use of genetically modified bacteria to combat the red imported fire ant is a potentially powerful tool. To reach this goal, we provide here some initial studies towards a possible field application for biological control of the fire ant. Firstly, bacteria which are closely associated with the fire ant midgut can be readily isolated and cultured *in vitro*. Secondly, a robust method for genetic transformation of these bacteria exists, and genetically modified bacteria can be stably maintained with minimal loss of the foreign gene both *in vitro* and *in vivo*. Lastly, genetically transformed bacteria can be successfully re-introduced back to the fire ant colony and can survive in the fire ant for at least 7 days.

Future Directions

One of the potential shortcomings of genetically modified bacteria generated using shuttle vectors is genetic instability and the subsequent loss of the plasmid transformation vector. To overcome this, we are utilizing a new approach involving the use of DNA integration elements that are compatible with bacteria. Future studies will focus on the utilization of *Himar1*, a *mariner* family transposable element that was originally identified from the horn fly, *Haematobia irritans*, for integrative transformation.

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Range Expansion of Two Species of *Pseudacteon* (Diptera: Phoridae) in Alabama

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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 ant occur in Alabama. The red imported fire ant, *Solenopsis invicta,* is located in the southern portion of the state and the black imported fire ant, *Solenopsis richteri*, is located in northwest Alabama.

Although the black imported fire ant was introduced into the United States before the red imported fire ant, its current range is thought to be northeastern Mississippi and northwestern Alabama. Vander Meer et al. (1985) first detected a hybrid between the two species in Mississippi. The hybrid is thought to populate the northern tier of Alabama, Mississippi and Georgia.

Phorid flies (Fig. 1) in the genus *Pseudacteon* have shown some promise in the battle against fire ants (Porter 2000). Currently, eleven populations of phorids have been successfully established in Alabama (Fig. 2). *Pseudacteon tricuspis* shows a strong preference for *S. invicta* and is established at seven sites in South Alabama. *Pseudacteon curvatus* shows a strong preference for *S. richteri* and the hybrid fire ant. It is established at four sites in North Alabama (releases in Madison and Lauderdale counties by K. Ward).

Materials and Methods

Releases of *P. tricuspis* and *P. curvatus* were conducted as described by Graham et al. 2003. The first successful release was in 1999 and new releases have been conducted yearly in different counties (Fig. 2).

A release site and a corresponding control site approximately 9.5 km apart were selected in Macon and Talladega. In Houston, Lowndes, Walker and Cullman, these sites were ca. 32 km apart. Two plots were established at each site for bait stations and pit fall traps. Sampling areas for population data were set up in conjunction with each plot. Data collected in each sampling area were total number of mounds and mound size. At the Baldwin, Barbour, Marengo, and Tuscaloosa sites, *Solenopsis* spp. populations are monitored by counting mounds in three 0.1 ha circles near the release site.

Results

P. tricuspis reached the Macon control site in mid- to late-summer of 2000. *P. curvatus* were found approximately 1.6 kilometers north of the Talladega control site in August 2002, but were not found south of the site until 2004. Flies from the Houston and Cullman sites have not been found at the control sites. Flies have not been found at the Lowndes control site, but have been found several miles past it.

Mound data are presented from the three oldest sites in Fig. 3. Droughts occurred in Alabama in 1999, 2000, and 2001. In addition, the coldest November and December on record were recorded in 2000. These environmental factors have influenced fire ant populations, as evidenced by the low number of mounds in May 2001 in Macon and reduction in mound numbers in 2001 and 2002 at the Talladega control site, where no phorids had been found at that time. Mound data in Fig. 4 are from release sites with no corresponding control.

Except for the Lowndes Co. release site (Fig. 3), mound numbers appear to be decreasing at each site where phorids have been released. Phorids are now present at all controls shown in Fig. 3 and mound numbers appear to be decreasing at these sites also.

The two *Pseudacteon* spp. released in Alabama have been recovered at eleven of twelve release sites and are spreading rapidly across the state and into Georgia (Fig. 5). These populations were mapped extensively during the 2004 field season. The ovals estimate the currently mapped ranges of the eleven populations (Fig. 5). The ranges of the Macon and Talladega populations are underestimates. We have not found the leading edges of these two populations.

P. tricuspis and *P. curvatus* have expanded their ranges to cover over 35,000 sq. miles in Alabama and Georgia. *P. curvatus*, which prefer *S. richteri* and the hybrid fire ant, are now moving into populations of *S. invicta* (see green squares, Fig. 5) and we expect to find *P. tricuspis* on hybrid fire ant populations soon.

Even though it appears that the phorid flies may have affected the fire ant populations at these sites, further study will be required to determine if these population reductions are permanent and due to the flies and not other factors.

Sites in the area where *P. curvatus* and *P. tricuspis* now coexist have been monitored since 2003, when no flies were present. Hopefully, the presence of more than one species of these parasitoids in an area will reduce fire ant numbers more dramatically and permanently than the reductions seen in Figs. 3 & 4 and we will be able to document the reduction in fire ant mounds.

Acknowledgements

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Fig. 1 Phorid fly attacking fire ant worker. Photo courtesy of S. Bauer.

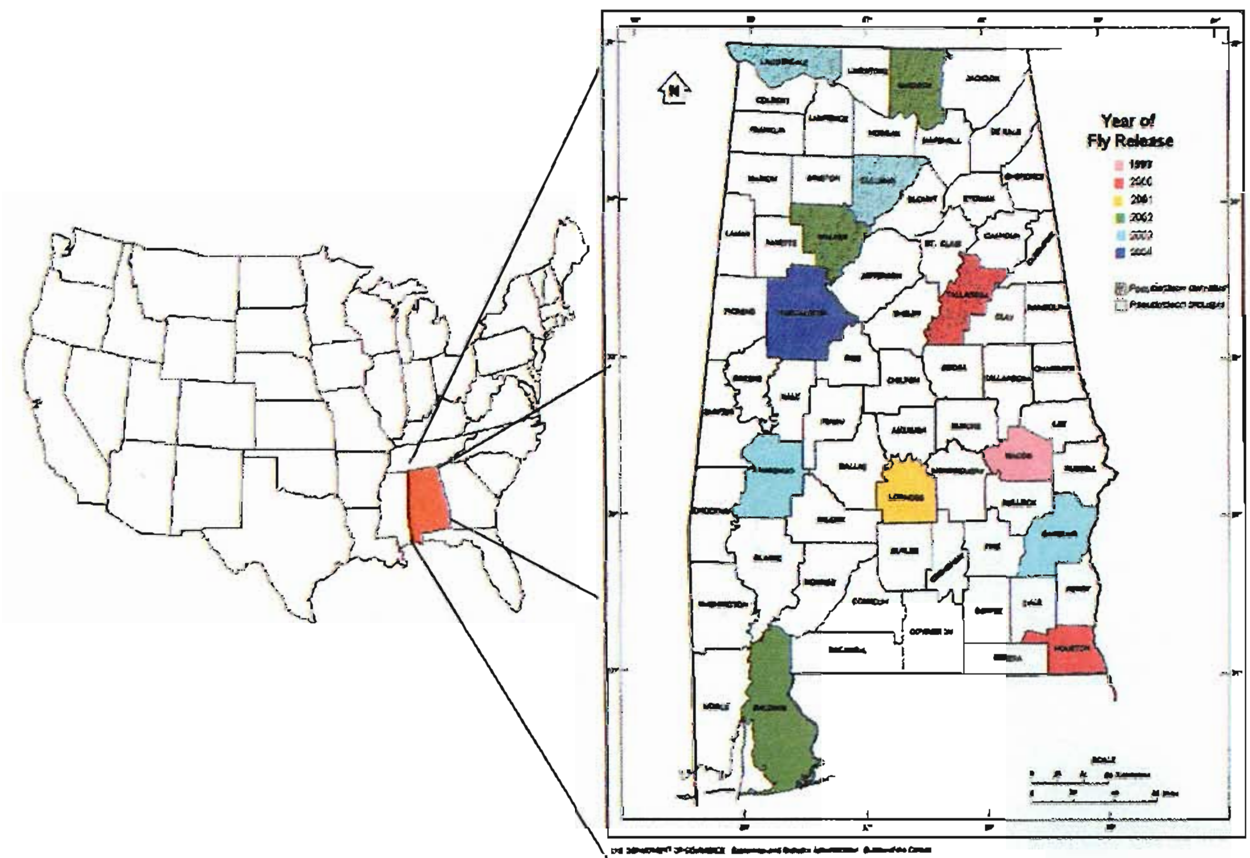

Fig.2 Counties where phorid flies have been released in Alabama. The year of the release is noted in the legend.

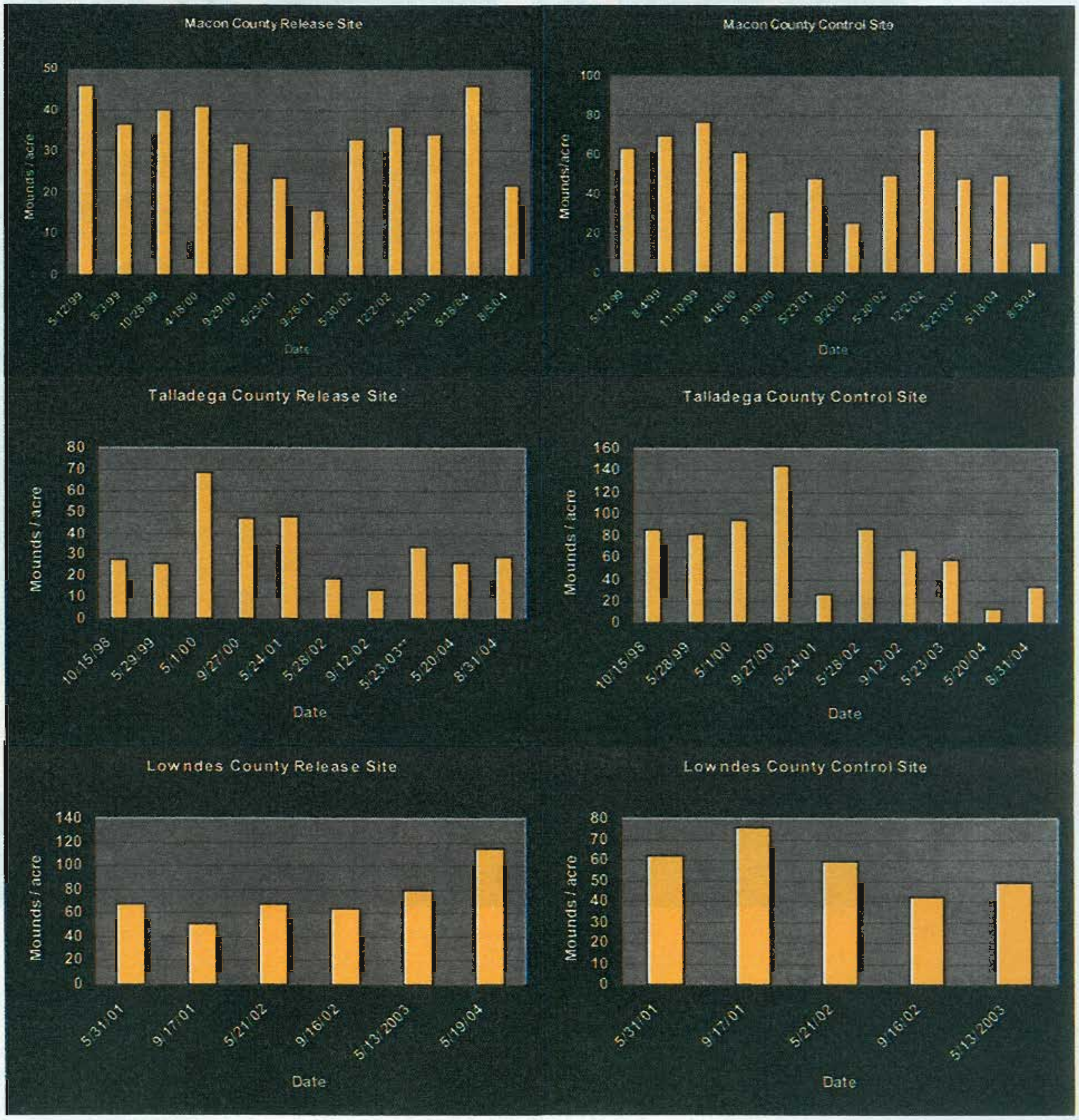

Fig. 3 Fire ant population data from the first three phorid releases and corresponding release sites.

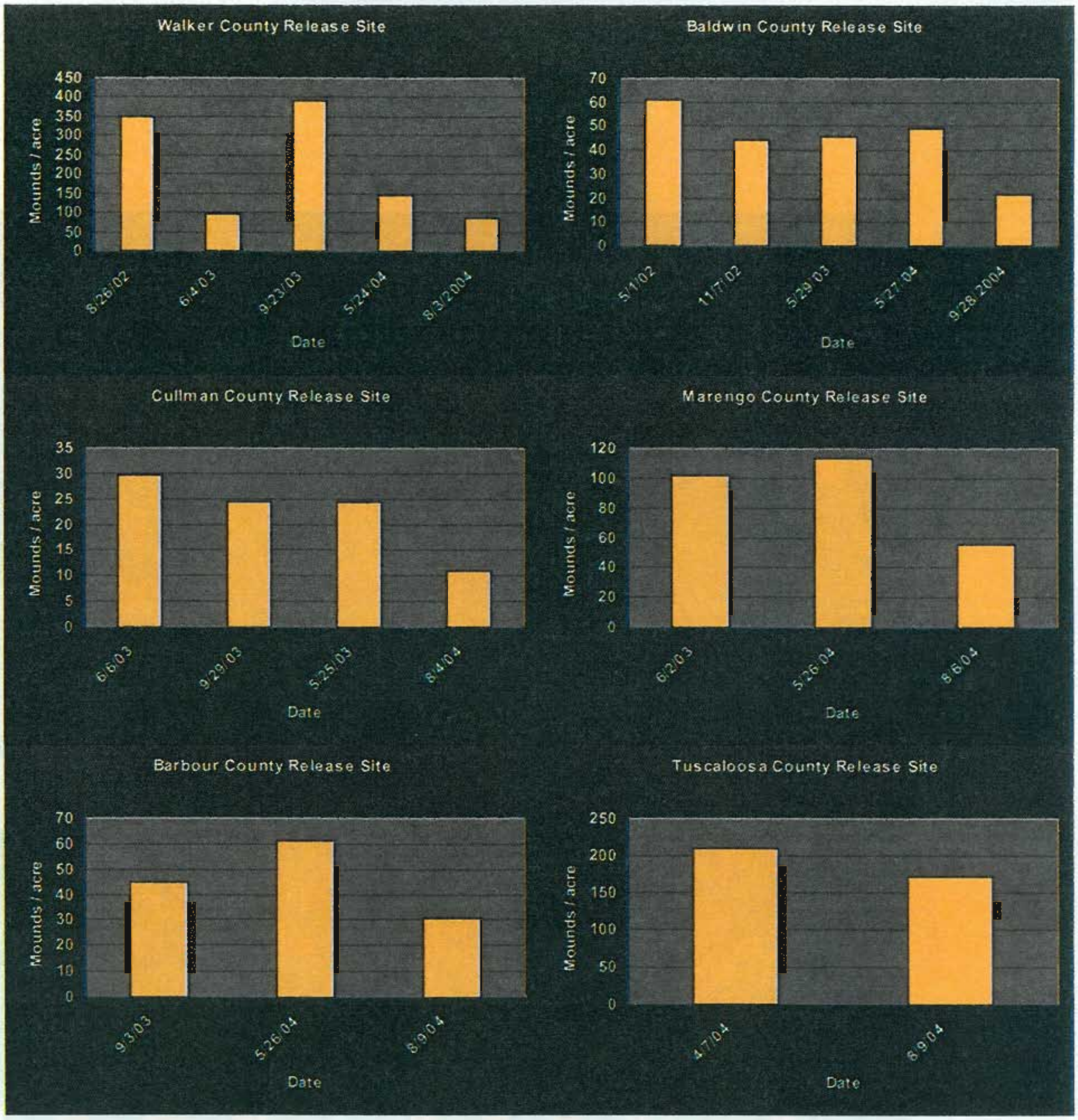

Fig. 4 Fire ant population data from release sites only. First data bar on each graph corresponds with phorid fly release date.

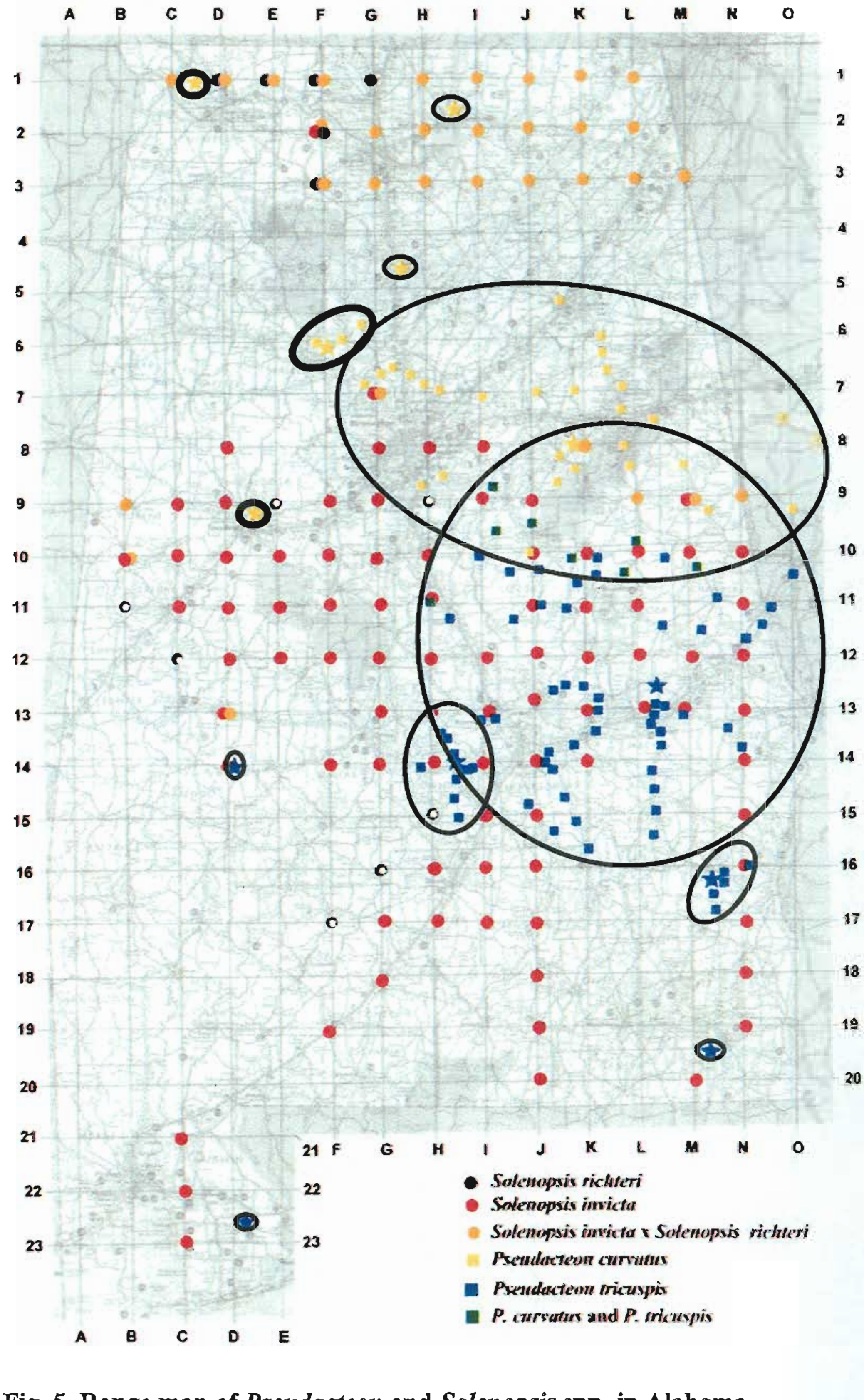

Fig. 5 Range map of *Pseudacteon* and *Solenopsis* spp. in Alabama

Host Range of the Microsporidian Pathogen *Vairimorpha invictae* at Three Field Sites in Argentina

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The microsporidian pathogen *Vairimorpha invictae* is being evaluated for possible release in the United States as a potential classical or self-sustaining biological control agent for imported fire ants. We examined the host range of this pathogen in northern Argentina by collecting ants and other arthropods from three sites where *Solenopsis invicta* fire ant colonies had high levels of infection (28-83%). We examined 235 non-ant arthropods from 10 orders, 43 families, and more than 80 species with a PCR screening procedure; none were infected with *V. invictae*. We also examined 509 non-*Solenopsis* ants from 12 genera, 19 species, and 61 baits using the same PCR screening procedure; again, none were infected with *V. invictae*. These data indicate that, in its native South American range, *V. invictae* is specific to *Solenopsis* fire ants.

Revision of the South American Thief Ants of the genus *Solenopsis* (Hymenoptera: Formicidae): A potential biological control agent for the red imported fire ant

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Background:

The red imported fire ant, *Solenopsis invicta,* was introduced into the United States over 60 years ago and since then has spread quickly. An effective treatment is needed and biological control with other ant species may be the appropriate path to take. However, the systematics of New World ants was largely accomplished more than a hundred years ago. Descriptions of species and delineation of species complexes were based on few characters. This system, practiced by specialists who seldom communicated and exchanged specimens, produced a confused jumble of names with many trinomials and quadrinomials. Moreover, traditional ant systematics has been mainly based on morphological characters, such as body structure, hair, etc., which may cause confusion and misclassification. Consequently, efforts at effective identification of many New World ant species are extremely difficult. Advances in the field of molecular systematics have helped to rectify many of these classification errors. A good example is the thief ants of the genus *Solenopsis.* Many species of this group live near nests of other ants, where they steal food and brood from these hosts (Fig. 1). They also appear to be effective predators, and may be natural enemies of the founding queens of the imported red fire ants; *Solenopsis invicta* (Lammers, 1987; Pacheco, personal data; Mackay, personal data) (Fig 15).

Significance:

- The systematics of *Solenopsis* is of particular importance because *Solenopsis invicta* has emerged as a significant pest.

- Thief ants kill founding queens of *Solenopsis invicta* but few people can identify these potential biological control species.

- It is crucial to determine the relationships among these ants to attain a revised and functional taxonomy.

Difficulties to Taxonomists:

- Tiny ants– the largest are only 2 mm in total length.
- Cryptic species—many with very similar phenotypes.
- Inconspicuous and difficult to capture—many are subterranean or nest in twigs.

Thief Ants:

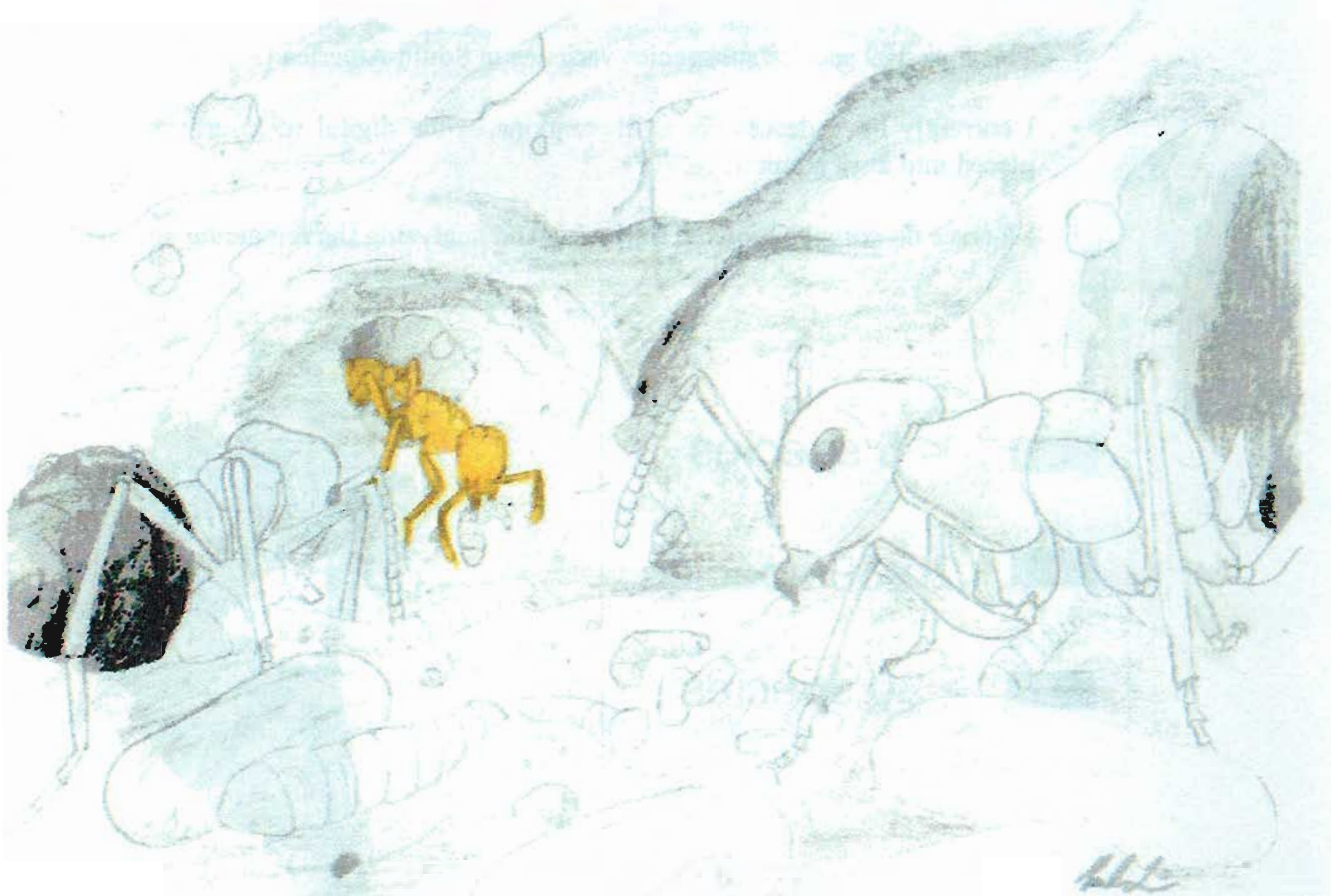
- The thief ants were originally proposed as the subgenus *Diplorhoptrum* of *Solenopsis* by Mayr in 1855.
- In 1968, Baroni-Urbani raised *Diplorhoptrum* to the status of a genus; later Kempf (1972) again synonymized it with *Solenopsis*, where it has remained (Bolton,1987).
- Creighton (1930) first attempted to delineate species complexes in the thief ants, based on unspecified "characteristics that are fairly consistent."
- Moreno (2001) reorganized the species complexes into 5 groups.
- Both focused on North American species.

Species Complexes:

The following 7 species complexes are defined:
1) *wasmanni*, 2) *brevicornis*, 3) ***fugax***, 4) ***molesta***,
5) *pygmaea*, 6) *stricta* and 7) *succinea.*

Identification keys, descriptions, and illustrations to each have been developed.

Figure 1: Thief Ants pilfering:



S. molesta pilfering brood from a *Formica sp.* nest.

Hypothesis 1

- Thief ants can be categorized into species complexes based on morphological variation.

Specific Aim:

- Revise the S.A. thief ants of the genus *Solenopsis*.

Materials and Methods:

- Obtain field and museum collections.

-"classical" and unidentified collections

- Re-organize and group species into complexes.

- Create keys to species including illustrations and photographs (species and labels).

Preliminary results:

- There are 129 species/subspecies/varieties in South America (Fig 2).
- I currently have descriptions, illustrations, some digital micrographs, and have placed into keys about 55%.
- I foresee no complications in retrieving and analyzing the remaining species.

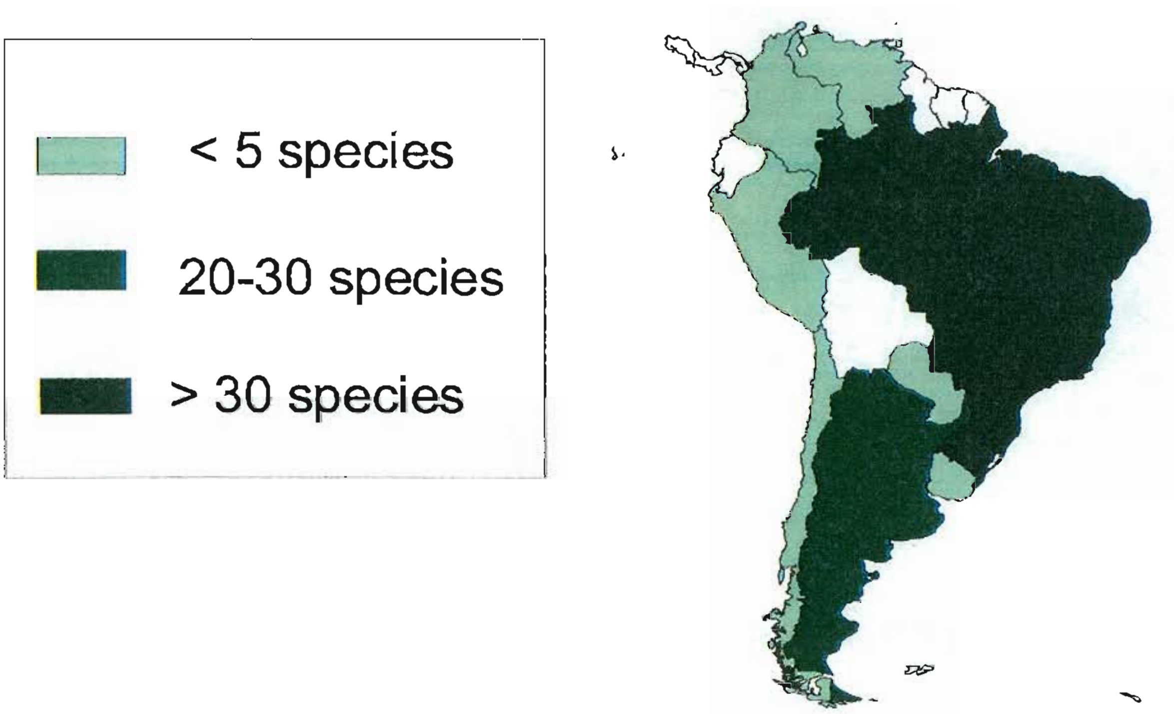


Figure 2: Collection bias in South American countries. There is a total of 129 species in South America and 47% are found in Brazil (26%) and Argentina (21%). This map is based on 60/129 species.

fugax species complex:

Diagnosis:

- The dorsum of the head is coarsely punctate (punctures larger than the diameter of the hair arising from them).

- There are usually four teeth on the anterior border of the clypeus (Fig. 3).

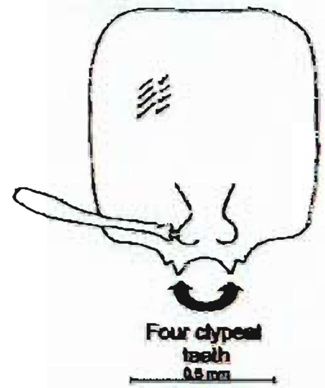
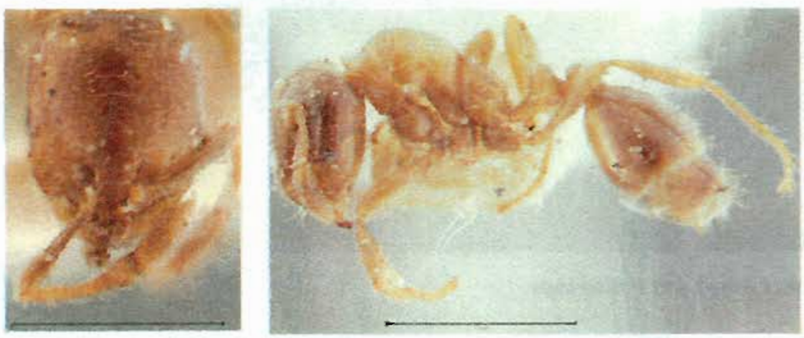
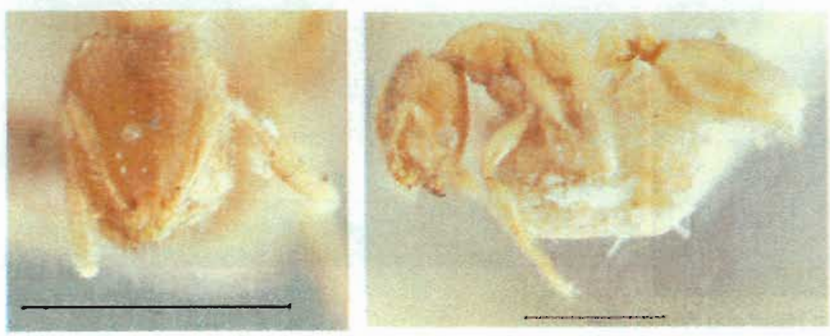


Figure. 3

Digital micrographs (José Pacheco):



Figures. 4 & 5: *S. germaini* worker; Head and Profile view.



Figures. 6 & 7: *S. patagonica medeis* worker; Head and Profile view.

molesta species complex:

Diagnosis:

- Most species in this complex are small-to medium-sized, yellow ants, although a few species are small, black ants.

- There are only two clypeal teeth along the anterior border, occasionally two swellings (rarely developed into teeth) are present at the position of the extralateral teeth (Fig. 9).

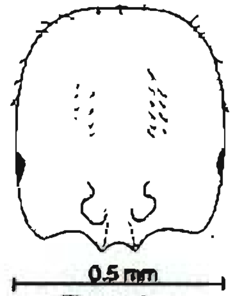
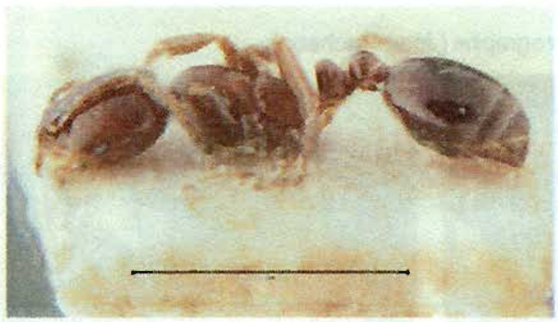


Figure. 9

Digital micrographs (José

Figures. 10& 11: *S. weiseri* worker; Head and Profile view.

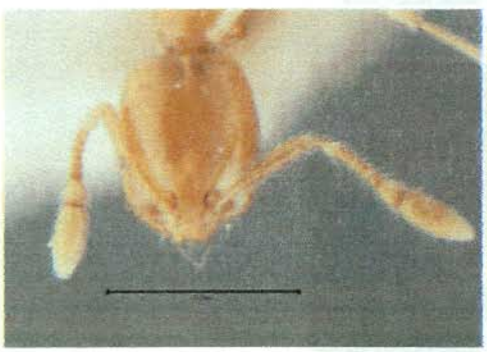
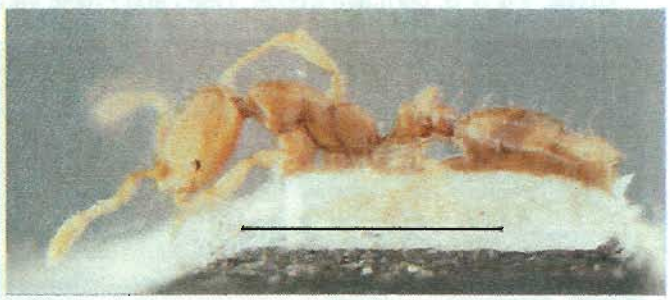



Fig. 12 & 13: *S. franki* worker; Head and Profile view.

Hypothesis 2:

- Evolutionary relationships can be determined using morphometrics, coded morphological characters, and mtDNA sequences.

Aims:

- To determine the phylogenetic relationships of the S.A. thief ants based on measurements of phenotypic characters of all species. Characters will be polarized for all species using outgroups from within and outside the genus.

- The purpose is to create cladograms that reveal evolutionary relationships among the thief ants.

Methods: Morphometrics:

- Using a Wild dissection microscope (80X) with micrometer:

TL, HL, HW, etc. (16 total).

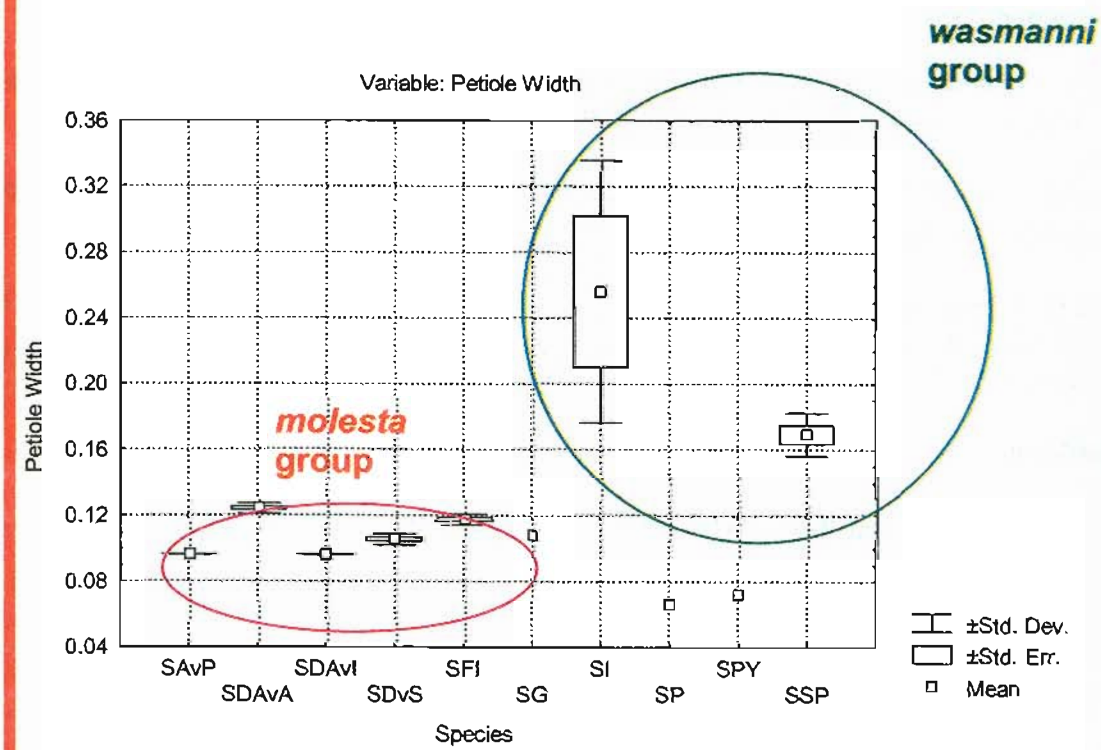
- Measuring types as well as replicates

(within and between variation).

Statistical Analysis:

- Basic statistics (Fig. 8): power test, Principal Component Analysis, Cluster analysis, Systat 10.1 and Statistica 5.0 software programs.

Figure 8: Box and Whisker Plots of Petiole Width for 10 *Solenopsis* species (n = 34)



Methods: Phylogenetic Analysis:

A) Morphological Analysis:

Thief ants are more derived than fire ants
Solenopsis invicta and the closely related *Monomorium minimum* as outgroups.

Coded characters: (0, 1, 2, etc.)
clypeal teeth, head shape, propodeal sculpture, etc.
(27 total).

PAUP*:
Hypotheses of phylogenetic relatedness.

B) Molecular:

- Wetterer *et al.* (1998) investigated the phylogeny of fungus growing attine ants using mitochondrial sequence data as well as traditional morphological techniques.

- Kronauer *et al.* (2004) estimated the phylogenetics of the honey pot ants (genus *Myrmecocystus*) based mitochondrial DNA sequences.

- Mitochondrial cytochrome oxidase (CO) I and II genes provide good phylogenetic resolution of genera and species of the tribe Attini (Wetterer *et al.* 1998).

- CO I and II have also been successfully employed in other lower level phylogenetic studies of Hymenoptera and other insects (e.g., Willis *et al.* 1992, Spicer, 1995).

Hypothesis 3:

- COI sequences will be useful in distinguishing between *Solenopsis* species.

Aim:

- To sequence a segment of the mitochondrial DNA of a variety of thief ant species. COI will be sequenced to compare and distinguish between species. Additionally, outgroups will be sequenced in order to determine phylogenetic relationships.

Methods and Materials:

- Using the polymerase chain reaction (PCR), I will amplify a segment of the mtDNA using ant primers "CI-13" and "CI-14" devised by Quek (pers. comm.) (Fig. 14).

CI-13 (5' to 3'): ATA ATT TTT TTT ATA GTT ATA CC

CI-14 (5' to 3'): GTT TCT TTT TTT CCT CTT TC

- The pair amplifies 565 bp of COI. Amplified products will be sequenced using established protocols. Sequences will be aligned using ClustalW.

- To infer relationships among the species of the genus, several phylogenetic analyses will be performed using PAUP* 4.0 b (Swofford, 2002), including a variety of model-based methods and maximum parsimony.

Preliminary:

- 57 species are available for molecular analysis. I currently have species from all 7 species complexes.

- Additionally, I have access to a large supply of both *Solenopsis molesta* (*molesta* group) and *Solenopsis tennesseensis* (*pygmaea* group) from El Paso to do the initial DNA work and optimize the protocols.

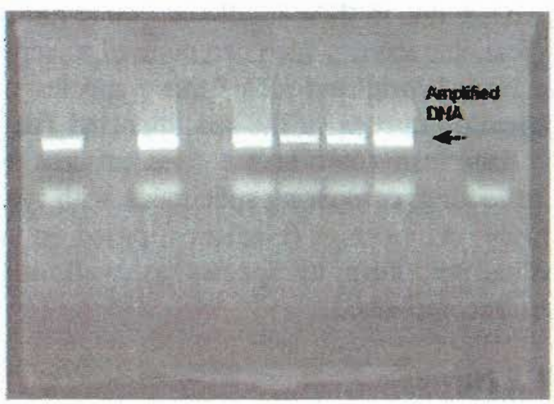
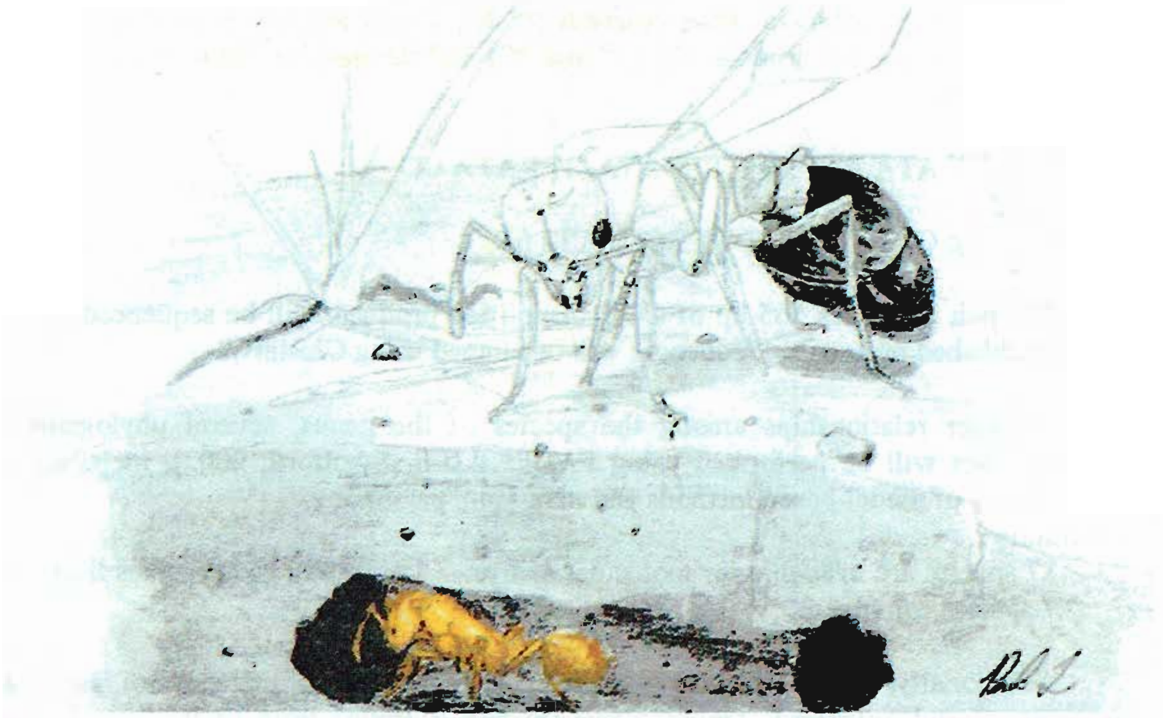


Figure. 14. Visualization gel of JP1-PCR.
The bands indicate a successful amplification of DNA.

Fig. 15: Founding queen of *S. invicta*.



Thief ants kill the founding queens of the red imported fire ant.

Summary:

Thief ants have been ignored, due to their small size (most rarely exceed 2 mm), and the lack of good morphological characters which can be used to separate species. A number of species occur in the New World and all of these species would be expected to be predators on the nest founding queens of the red imported fire ant. My study will be a revision of the South American thief ants. The process of the revision will be accomplished by field collecting, comparing specimens with types to verify their names, and determine evolutionary relationships (cladistics) based on morphometrics as well as molecular analyses. Moreover, keys to species as well as illustrations, and other identification aids will be accomplished.

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Dr. Swee Peck Quek, Dr. Gary Alpert, and Stefan Cover from Harvard University. A sincere thanks to Paul Lenhart for the illustrations.

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Distribution of Imported Fire Ant Populations in Alabama

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Introduction

The black imported fire ant, *Solenopsis richteri* Forel, was the first imported fire ant identified in the United States (Loding, 1929) and was originally referred to as *Solenopsis saevissima* (Fr. Smith). Wilson (1951, 1953) indicated that the population consisted of a "dark phase" for the first ten years or so that corresponded to the southernmost geographic variant of the South American population.

By 1957, Wilson and Brown reported a significant decline in the "dark phase" and an increase in the "light phase" in almost every locality except Lowndes and Noxubee Co. in Mississippi. In 1972, Buren's revision of the *S. saevissima* complex renamed the "light phase" *S. invicta* and the "dark phase" *S. richteri*. Range maps by Buren et al. (1974) show *S. richteri* located in north central to northeastern Mississippi and northwestern Alabama. More recent maps (Taber, 2000) show a similar range, but shifted somewhat further north and east, now extending to the Tennessee border.

Vander Meer et al. (1985) first detected a hybrid between the two species in Mississippi. Diffie et al. (1988) reported the hybrid from 10 counties in western Georgia and 22 counties in north central Alabama in surveys in 1985-6. Prior to this survey, we found the hybrid in Talladega County.

The purpose of this study is to determine the approximate location of each imported fire ant species in Alabama. This will allow us to release biological control agents on their preferred host.

Materials and Methods

A grid was superimposed on a map of Alabama. The grid is an extension of the one used by Diffie et al. (2002) in Georgia. The squares are approximately 27 km x 27 km. Worker ants were collected from three mounds at or near the intersection on the grid. These sites were surveyed during 2003 and 2004.

Ants were collected from each site by inserting a 30 x 80 mm plastic tube into a mound and capping it once at least 25 ants fell into the tube. Ants were chilled and approximately 25 were removed from the sample tube. These were placed into seven ml glass scintillation vials and covered with hexane. After 24 hours, the hexane was removed, added to a clean seven ml scintillation vial, and allowed to evaporate. These vials containing cuticular hydrocarbon residues from the ants were shipped to CMAVE in Gainesville, FL for species determination as described by Vander Meer et al. 1985.

Results & Discussion

Based upon collections in Georgia by Diffie et al. (2002), conversations with J. T. Vogt in Mississippi and our collections in Talladega County, we assumed that the southernmost line of the hybrid range would extend into central Alabama. Instead, the range appears to approach the central portion of Alabama from the north in the eastern (Randolph Co.) and the western (Pickens Co.) parts of central Alabama, but remains north of Birmingham in the middle of the state.

The southern two-thirds of the state appear to be infested primarily with *S. invicta*. One mound of the hybrid was found in Marengo Co. This was the only mound of the hybrid fire ant found south of Pickens Co.

S. richteri has only been found in the northeast and north central portion of the state, extending from near Huntsville to the west. One site near Courtland in Lawrence Co. had all three ant species at the site.

Buren (1972) speculated that the northward progression of *S. invicta* would be limited by winter kill conditions and that the species could be more successful in progressing northward in the eastern costal plans. It follows that *S. richteri,* which comes from cooler regions in S. America, and the hybrid fire ant would be more vigorous in cooler ecological regions here.

We plan to continue mapping the ranges in the northern third of Alabama this summer. Data from this study will add to the current range information collected by Diffie in Georgia and Streett in Mississippi. It will also allow us to improve our biological control release protocols and to ensure that we release the correct biological control agent on the correct fire ant species.

Acknowledgements

The authors wish to thank Catherine Preston for laboratory analysis and Justin Mims, David Findlay, Christina Wolf, Brad Bennett, Jesse Stalons and April Dove for assistance collecting samples.

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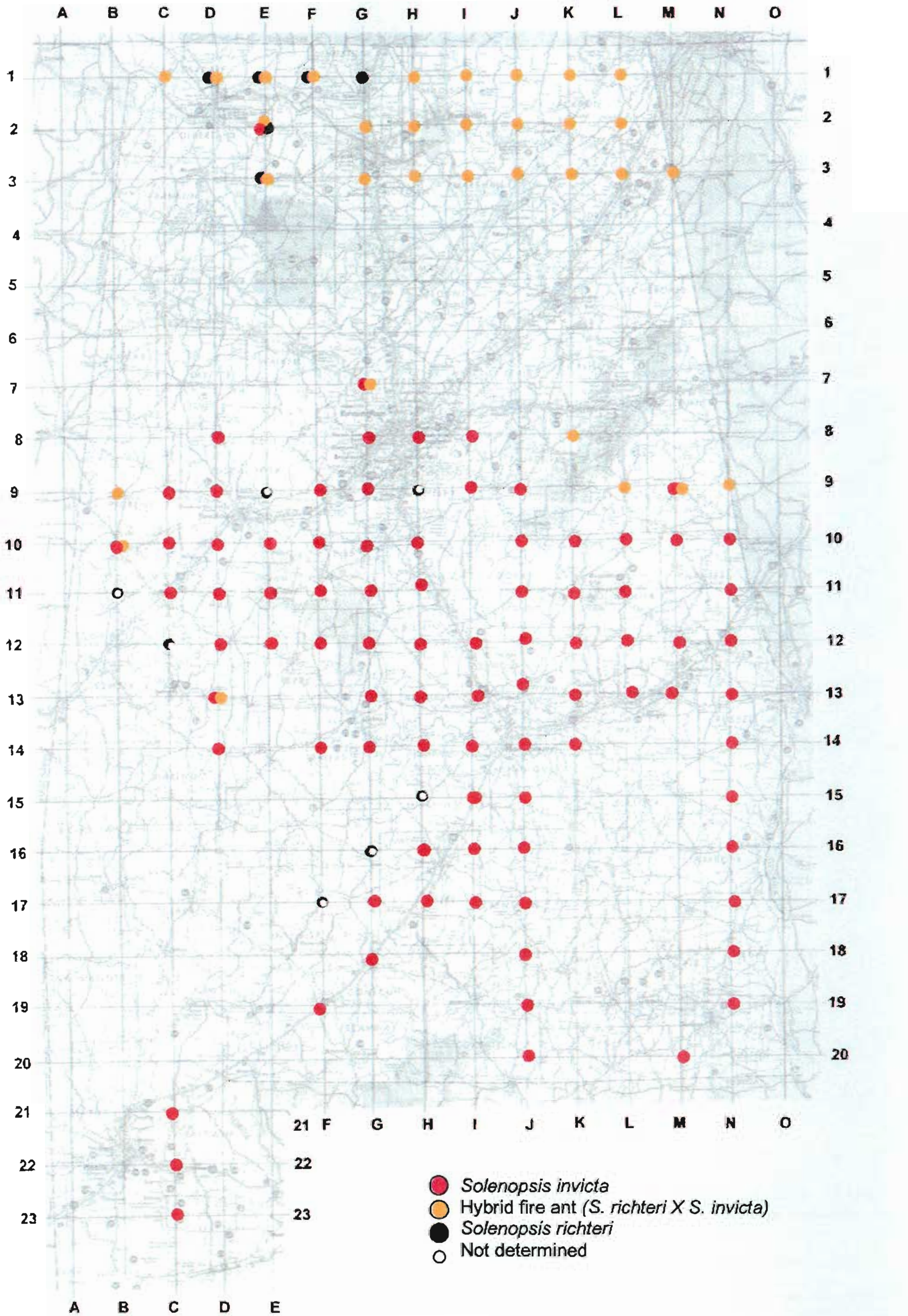
Grid Map


A B C D E F G H I J K L M N O
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Solenopsis invicta
Hybrid fire ant (S. richteri X S. invicta)
Solenopsis richteri
Not determined

The effect on native ant biodiversity in Northern Mississippi by the Black Imported Fire Ant (*Solenopsis richteri*) Forel

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Abbeville, MS 38601

Introduction

Although most exotic species fail to establish or have minor effects on their host communities (Elton 1958; Simberloff 1981), some invasives can dramatically alter community structure (Mooney and Drake 1986). Since its introduction, the Red Imported Fire Ant, *Solenopsis invicta,* is widely believed to suppress native ant diversity. It shows many of the biological properties of a weed and is adapted to the opportunistic exploitation of ecologically disturbed habitats (Tschinkel 1986). Polygnous RIFA forms have been shown to decimate indigenous ant fauna upon invasion (Porter and Savignano 1990). However, Rao and Vinson (2004) showed that native ants may not be completely defenseless to the fire ant, *S invicta's* onslaught and could provide some level of competitive exclusion to smaller *S. invicta* colonies.

At the University of Mississippi Field Station (UMFS), we investigated the effect on native ant biodiversity by the Black Imported Fire Ant (BIFA), *Solenopsis richteri*. This invasive species, a monogyne congener of the more widespread *Solenopsis invicta*, is now limited to a range of Northern Mississippi, NW Alabama and Southern Tennessee. The UMFS, located in Holly Springs National Forest, provides an excellent opportunity to investigate its ecological role. The objective of this study is to provide a quantitative understanding of the biodiversity of native and imported ants in distinctly different ecological settings (disturbed vs. undisturbed communities). We expect *S. richteri* to be the most common and dominant ant encountered in both pitfall and baited samples in disturbed areas.

Materials and Methods

Four sites were utilized at the University of Mississippi Field Station to compare differences in ant biodiversity (sites 1 and 3 as disturbed and 2 and 4 as undisturbed). Site 1 was a *Fescus* dominated field located near the Turkey Aviary at the UMFS. It was mowed twice during the sampling period to simulate a disturbance regime. Site 3 was located in a drainage field 0.4 km southeast of the UMFS main building. Site 2 was a mixed hardwood forest located 15 yards west of ponds 1-3 at the UMFS. Site 4, also a mixed hardwood forest, was located 10 yards east of the DTTB at the UMFS. Sites 1 and 3 contained 40 and 10 located *S. richteri* nests respectively, whereas sites 2 and 4 had 2-3 nests. Sampling began on 29 March 2004 and concluded on 19 July 2004; pitfall traps and baits were placed every two weeks (sampling methods alternated each week). Twenty pitfall traps were placed 10 m apart on a 100 m transect and collected after 48 hours. All collected ants were placed in 95 % ethanol and identified to the species level. Ten baits (5 with peanut butter and 5 with tuna) were randomly placed along two parallel 50 m transects at each site. Approximately 1 cm^3 of bait substance was placed on a 5 x 5 cm cardboard square and collected after a 1.5-hour interval. Baiting was conducted between 0900 and 1100 or 1500 and 1700 on sampling dates. All collected ants were placed in 95 % ethanol and identified to the species level.

Biodiversity was determined using three diversity measurements: species richness, Shannon-Weaver Diversity Index, and the Bergey-Parker Dominance Index. Species richness was defined as the number of species present at each site. The Shannon-Weaver Diversity Index (H') was used to measure species richness with respect to the dominant species at each site. Higher (H') means greater diversity at the site. The Bergey-Parker Dominance Index (D) was used to quantify the dominance of one to two species at each site. Higher (D) means greater dominance by one or two species at the site.

Results

A total of 2543 ants were collected from Site 1 and 3 pitfall traps. *S. richteri* made up 72% of the 1535 total ants captured in Site 1 and 38% of the 1008 total ants captured in Site 3 (Fig. 1; a full list of species collected by site is located in appendix 1).

Monomorium minimum (19 %) and *Dorymrymex bureni* (26%) were the next most common ants encountered at Site 1 and 3, respectively (Fig. 1). *Paratrechina vividula* was present at both sites, but was less frequently encountered at Site 1 (3%) versus Site 3 (18%). In the baiting experiment, 2851 and 726 ants were collected from Site 1 and 3, respectively. The two most common species collected in the pitfall traps were also encountered in the baiting trial; however, *M. minimum* was much more frequent (86%) than *S. richteri* (14%). Similar results were seen in Site 3 with *D. bureni* collected 74% of the time, with *S. richteri* (25%) and *P. vividula* (1%) far less frequent.

A total of 912 total ants were collected from pitfall traps at Sites 2 and 4. *Prenolepis imparis* was the most common ant collected in Site 2 (62%), whereas several different ant species were collected with similar frequencies in Site 4 (Fig. 2; a full list of species collected by site, is located in appendix 1). In the baiting experiment, 506 and 590 total ants were collected from Site 2 and 4, respectively. *P. imparis* (35%), *Crematogaster ashmeadi* (20%), and *Aphaenogaster* f/r/t group (18%) were the most frequent ants encountered at baits in Site 2. *Aphaenogaster treatae* (29%) was the most common ant collected at baits in Site 4 with several other ant species collected at similar frequencies.

Species richness was highest at Site 4 (23) and lowest at Site 3 (15) for pitfall traps (Table 1). Species richness was also high for Site 4 (14) in the baiting trials, followed by Site 2 (11), Site 3 (3) and Site 4 (2). Site 4 showed the highest Shannon-Weaver Diversity at 1.091624, with Site 1 showing the least at 0.413393 (Table 1). Site 1 showed a high level of dominance by *S. richteri* (D = 0.721824), whereas Site 2 showed a high level of dominance by *P. imparis* (D = 0.700709) (Table 1). Site 4, however, showed no particular dominance by any species (D = 0.164038) (Table 1). Both *M. minimum* and *D. bureni* showed high levels of dominance at both disturbed sites [D = 0.879691 (1) and D = 0.792011 (3)] in baited trials, whereas ant species in the undisturbed sites showed low dominance scores (Site 2 = 0.426877 and Site 4 = 0.271186).

Conclusions

Even though there is no significant difference in species richness between sites, *S. richteri* make up nearly 70 % of the total ants sampled in both Sites 1 and 3, but fewer than 7 % of the total ants sampled in Sites 2 and 4 in pitfall traps. Many of the species encountered in Sites 1 and 3 were rare and infrequently collected, whereas Sites 2 and 4 showed a more balanced distribution of native ants collected (Fig. 1 and 2). Furthermore, only two sampling techniques were used (baiting and pitfall traps). Hand collecting and leaf litter samples may have procured more species in the two undisturbed sites, increasing their species richness and diversity scores.

Despite its apparent numerical advantage, *S. richteri* was out-competed to baits by the ants, *M. minimum* (Site 1) and *D. bureni* (Site 3), at both disturbed sites. Over 7 times as many *M. minimum* (2508 vs. 343) and over 3 times as many *D. bureni* (575 vs. 174) were collected over *S. richteri* at baited samples. As Rao and Vinson (2004) showed native ants are able to compete and destroy small colonies of *S. invicta*. Colonies of *M. minimum* and *D. bureni* were typically seen in close proximity to relatively large *S. richteri* colonies and appeared unaffected by these large colony mounds. Perhaps both of these native ants are able to locate and protect food sources better than *S. richteri*, without being displaced by the BIFA. An investigation into the foraging strategies of these three ants could help explain these findings.

From this study it is unclear whether *S. richteri* is suppressing native ant diversity or if it is merely maximizing its opportunities within a disturbed community. Further investigation into the behavioral interactions occurring at baits and extensive sampling of both species number and abundance would better illustrate the effect of *S. richteri* on native ant diversity. Comparing native ant diversity and abundance between disturbed areas with or without *S. richteri* nests, may help better understand its impact on native ant populations and diversity.

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Table 1. Species richness, Shannon-Weaver Diversity Index (H'), and Bergey-Perker Dominance Index (D) scores for pitfall traps at all four sites (DS. = Disturbed site and US = Undisturbed site).

Site	**Species Richness**	**H'**	**D**
Site 1 (DS)	18	0.413393	0.721824
Site 2 (US)	19	0.700709	0.615108
Site 3 (DS)	15	0.685721	0.367063
Site 4 (US)	23	1.091624	0.164038

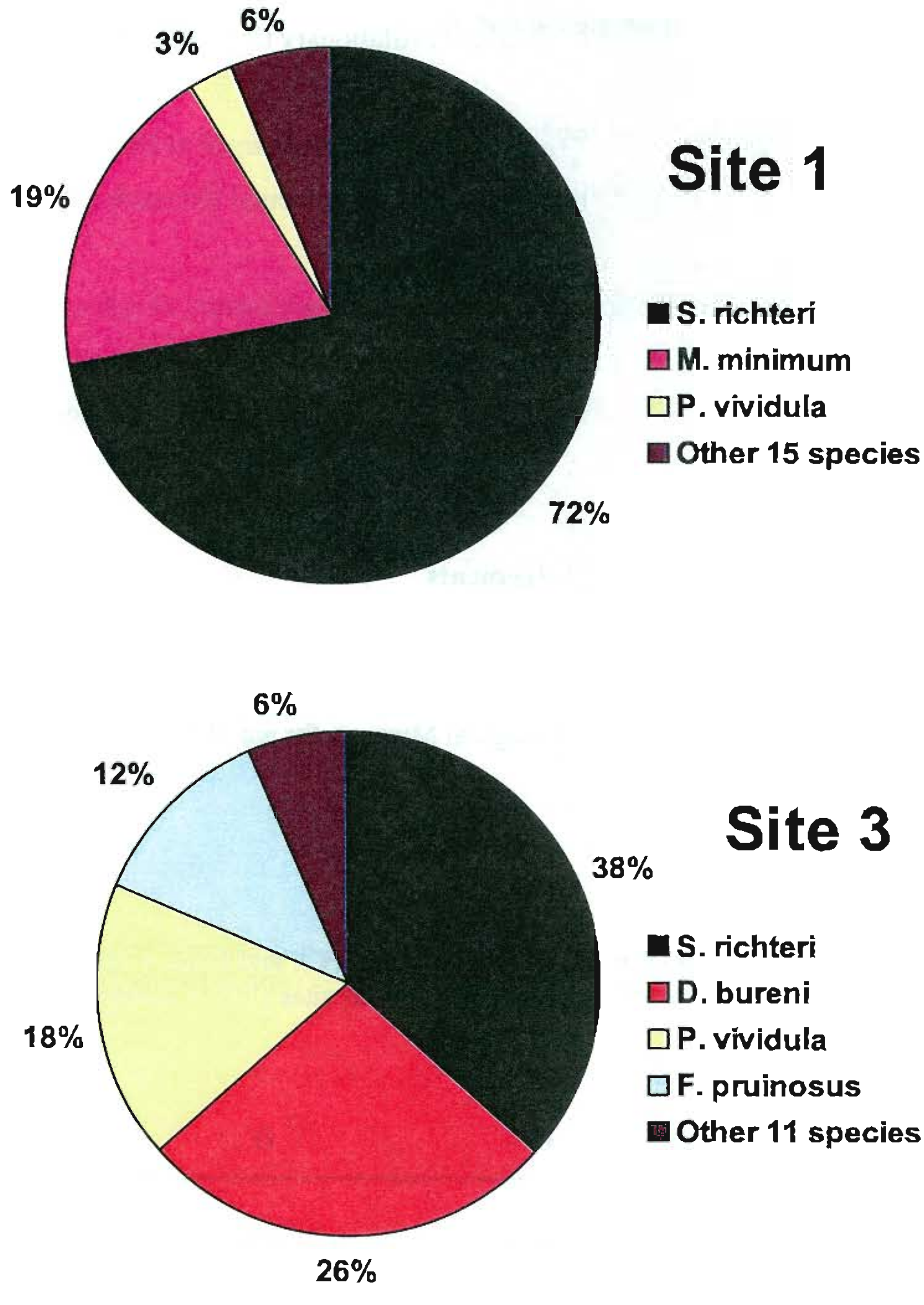

Figure 1. Percentage (%) of ants collected with pitfall traps in disturbed Sites 1 and 3.

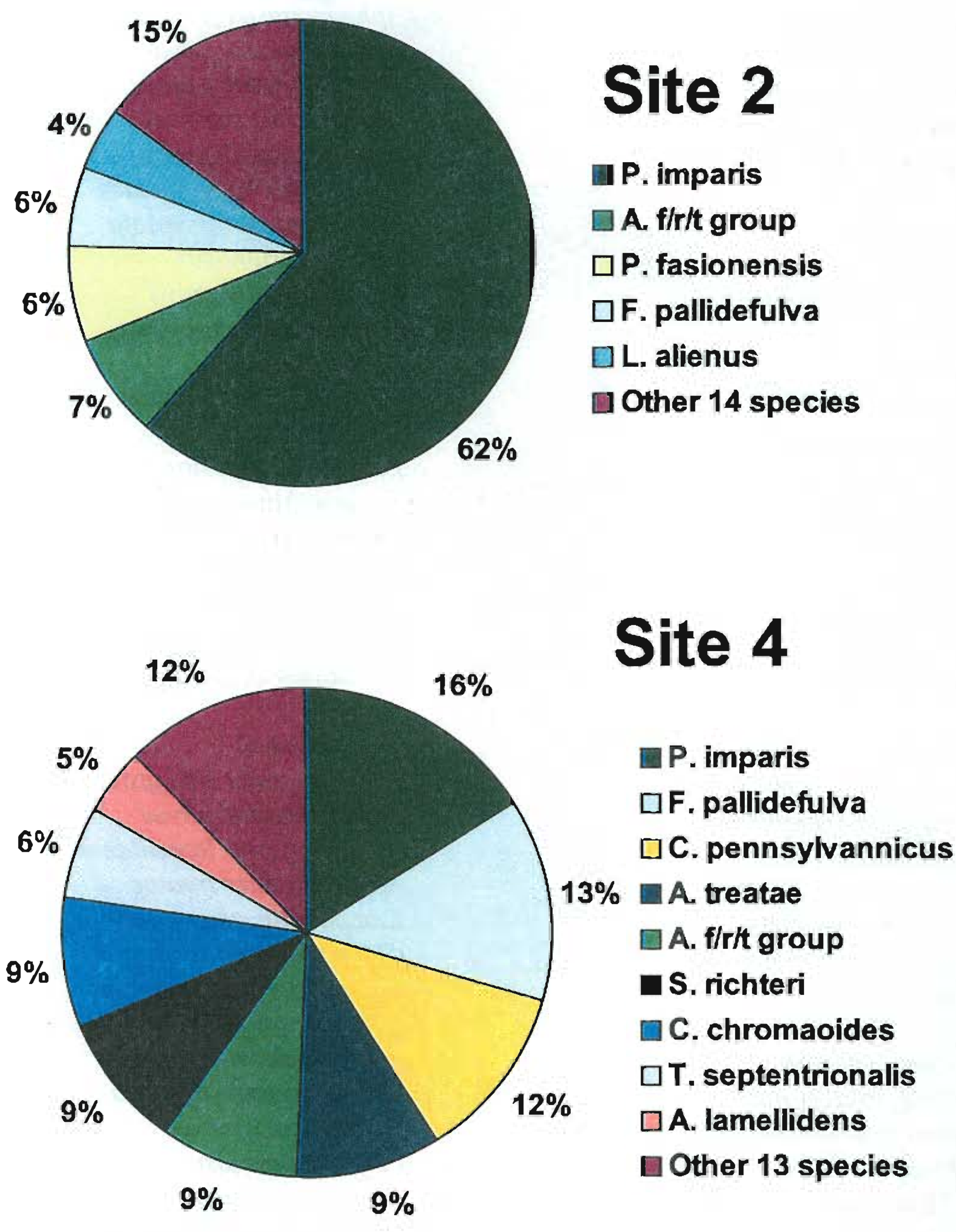

Figure 2. Percentage (%) of ants collected with pitfall traps in undisturbed Sites 2 and 4

Appendix 1. Species list for each site collected (both pitfall and baited trials).

Site 1

Aphaenogaster lamellidens
Aphaenogaster treatae
Camponotus americanus
Camponotus chromaoides
Crematogaster ashmeadi
Dorymyrmex bureni
Forelius pruinosus
Formica pallidefulva
Formica schautussi dolosa
Hypoponera opacior
Lasius alienus
Monomorium minimum
Myrmecina americana
Paratrechina vividula
Pheidole bicaranata
Pheidole dentata
Pheidole tysoni
Prenolepis imparis
Solenopsis richteri
Solenopsis molesta

Site 2

Aphaenogaster f/r/t group
Aphaenogaster fulvae
Aphaenogaster treatae
Camponotus castaneus
Camponotus chromaoides
Crematogaster ashmeadi
Crematogaster lineolata
Forelius pruinosus
Formica pallidefulva
Formica n. sp. *(pallidefulva)* group
Lasius alienus
Monomorium minimum
Myrmecina americana
Paratrechina fasionensis
Paratrechina vividula
Pheidole tysoni
Prenolepis imparis
Solenopsis richteri
Strumigenys louisianae
Temnothorax curvispinosis

Site 3

Aphaenogaster f/r/t group
Camponotus americanus
Camponotus chromaoides
Camponotus pennslyvannicus
Crematogaster ashmeadi
Crematogaster lineolata
Dorymyrmex bureni
Forelius pruinosus
Monomorium minimum
Paratrechina arenivaga
Paratrechina fasionensis
Paratrechina vividula
Pheidole bicaranata
Solenopsis richteri

Site 4

Aphaenogaster f/r/t group
Aphaenogaster fulvae
Aphaenogaster lamellidens
Aphaenogaster treatae
Camponotus americanus
Camponotus castaneus
Camponotus chromaoides
Camponotus pennslyvannicus
Crematogaster ashmeadi
Crematogaster lineolata
Dorymyrmex bureni
Forelius pruinosus
Formica pallidefulva
Formica schautussi dolosa
Myrmecina americana
Paratrechina vividula
Prenolepis imparis
Pheidole dentate
Solenopsis richteri
Stenamma meridionale
Temnothorax purgandei
Trachymyrmex septentrionalis

The Imported Fire Ant Mound in Three Dimensions

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INTRODUCTION

Imported fire ant mounds exhibit several characteristics that make them suitable targets for various remote sensing methods. Mounds have a characteristic elliptical shape, with the major axis roughly oriented in a north-south direction (Vogt et al. 2004). Other aspects of mound shape (centroid, slope angles, height) may ultimately prove useful for automated detection in remotely-sensed data, especially using distance-finding airborne devices such as LIDAR. This paper is a preliminary report describing the methodology we are using to obtain 3-dimensional data from fire ant mound surfaces in an attempt to extract additional morphological traits of mounds. The goals for the initial phase of this research were to (1) test the capability of a 3D scanner in the field, and (2) conduct preliminary analyses of mounds to determine the potential for creating unique classifiers for mounds based on their morphometrics.

MATERIALS AND METHODS

We are collecting data with a handheld laser scanning wand (Polhemus FastSCAN™ Cobra™, Polhemus, Colchester, VT) that casts a fan of laser light over the mound surface and simultaneously records the laser using a camera. An embedded FASTRAK® device records the position and orientation of the wand, resulting in a point cloud that can be exported in several data formats, smoothed, modeled as a 3-dimensional surface, and subjected to morphometric analyses. Preliminary data for three imported fire ant mounds located in Washington Co., MS were analyzed to determine whether it might be feasible to construct unique classifiers that would be useful for automated classification of images. First, contour plots of the mounds were examined to determine the approximate location of the mound apex. Next, mound symmetry was visually evaluated using plots of symmetry about the first moment; this was accomplished by plotting rotational slices of mean integrated square error about the first moment. This provided a measure of axial symmetry; any deviation from a straight line in a plot represents a departure from normality.

RESULTS AND DISCUSSION

The 3D scanner employed in this study proved useful for obtaining data under field conditions (Fig. 1), with some limitations. First, it was useful to shade the mound surface with a surveyor's umbrella and reduce reflected light at ground level by wrapping a large tarp around the sides of the umbrella. Trimming vegetation back at the mound periphery prevented spikes (outliers) in the resulting point cloud. A light sprinkling of talcum powder was useful for scanning areas of the mound that were difficult to image; in preliminary work, other coatings such as spray paints were also suitable for this.

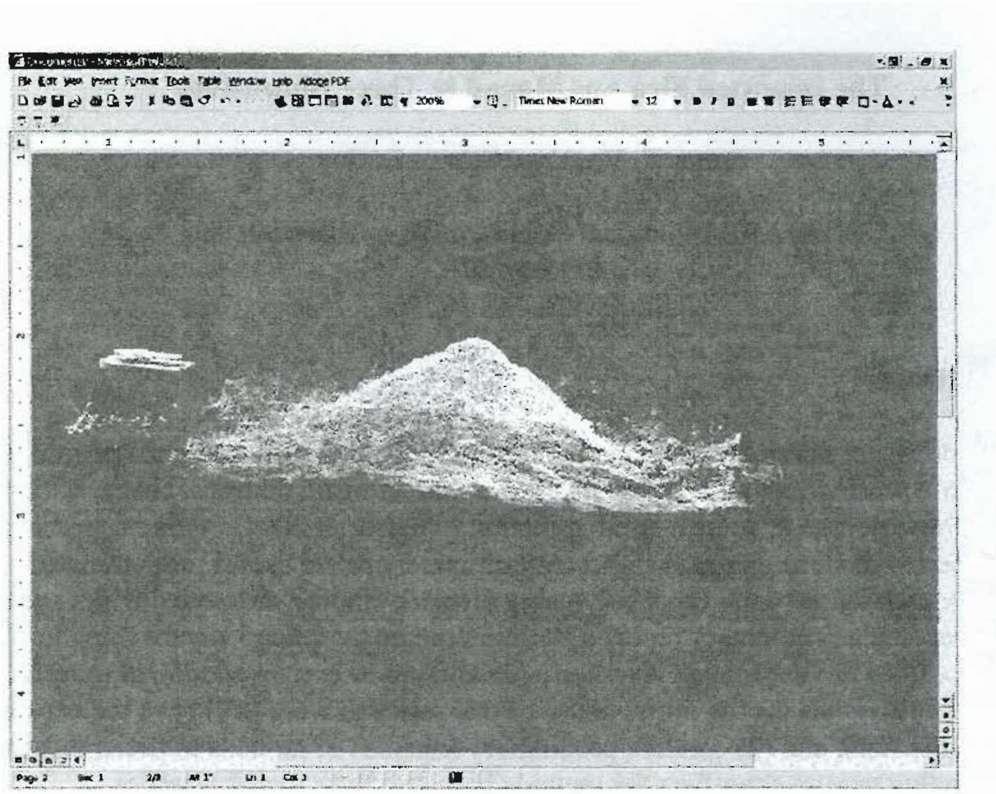

Figure 1. Screen shot of point cloud generated by 3D scanner. The arrow is a small wooden arrow used to orient the image to the north.

Visual examination of contour plots of mounds (Fig. 2a) confirmed that the apex of the mounds was generally north of center, with the major axis of the mound roughly oriented in a north-south direction. Finally, an examination of plots of symmetry (Fig. 2b) revealed consistent deviations from normality based upon rotational slices of mean squared error through the first moment.

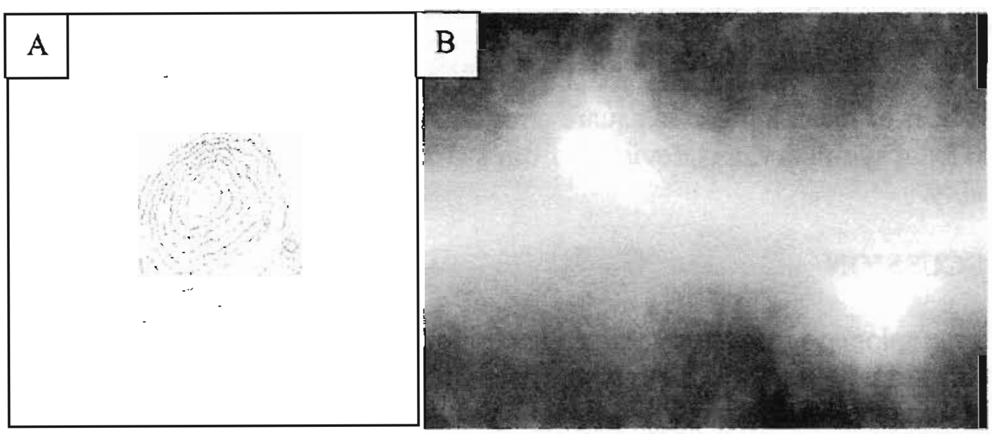

Figure 2. Contour plot of mound (A) and plot of symmetry about the first moment (B) from a three dimensional point cloud of an imported fire ant mound in the field.

Consistent departures from normality in three-dimensional scans of imported fire ant mounds indicate that it will be possible to derive unique classifiers for discerning mounds from other landscape feature and creating algorithms for automated detection of mounds. The methodology described in this paper will allow us to gather 3-dimensional mound data during different seasons and at different geographic locations for hypothesis testing and image classification.

ACKNOWLEDGMENT

We thank Mr. Bradley Wallet, Automated Decisions, LLC, for assistance with IDL programming and visualization.

REFERENCE CITED:

Vogt, J. T., D. A. Streett, and D. Boykin. 2004. Seasonal characteristics of black imported fire ant (Hymenoptera: Formicidae) mounds in northern Mississippi pastures. Sociobiology 43: 513-522.

AGENDA

Tuesday, March 22

3:00 – 5:00 pm Registration – Lobby (Oasis Hotel)

6:00 – 7:30 pm Reception – Patio (Oasis Hotel)
***Inclement weather plan: Beach View room (Beach Tower)**

7:00 – 8:00 pm – Poster Setup – Gulfport Room (Beach Tower)

Wednesday, March 23

7:00 – 3:00 pm – Registration – Pre-function area Ballroom B&C (Beach Tower)

7:00 – 8:00 am – Poster Setup – Gulfport Room

7:30 – 8:15 am – Continental Breakfast – Ballroom B&C (Beach Tower)

8:15 Call to Order – Ballroom B&C (Beach Tower)

8:30 Welcome

8:30 – 9:15 Keynote address
Dr. Jerome Goddard - Mississippi Department of Health, State Medical Entomologist
Health Effects of Human-Fire Ant Interactions

Session: Medical

Moderator: Ron Weeks

9:15 – 9:30 G. Howell, R. deShazo, A. Yates, N.P.D. Nanayakkara, **R. Rockhold**, Fire ant venom alkaloids: Update on mammalian toxicities and clinical relevance

Session: Chemical Control

9:30 – 9:45 P. Nester, Above and beyond the community-wide control efforts for the red imported fire ant in the near-urban areas of Texas

9:45 – 10:05 T. Taylor, Up-date of RIFARID LLC's patented method for instant eradication of RIFA colonies

10:05 – 10:35 30 Minute break
Moderator: Karen Vail

10:35 – 10:55 B. M. Drees, B. Summerlin, P. Nester, E. Brown, M. Heimer, Utility boxes and pasturelands: Fire ant management product assessments for Arinix® and Esteem®

10:55 – 11:10 T. Rashid, K. Vail, P. Parkman, J. Oliver, Cool-season applications of baits and/or chemical drenches to individual mounds

11:10 – 11:25 D. Oi, Characterization of delayed toxicity in fast-acting fire ant baits

11:25 – 11:40 S. B. Vinson, A. Rao, P. Mokkarala, Effect of red imported fire ant baits on some of the non-target ants

11:40 – 1:00 pm Lunch
(on your own – return by 1:00 pm)

Session:
Regulatory/Extension/Prevention/Eradication

Moderator: A. Callcott

1:00 – 1:20 C. Brown, APHIS regulatory overview

1:20 – 1:40 S. James, J. Oliver, A. Callcott, M. Klein, J. Moyseenko, N. Youssef, L. McAnally, Immersion treatments for imported fire ant quarantine with consideration for Japanese beetle grubs

1:40 – 2:00 D. Thompson, A. Jacobson, Can we establish guidelines to distinguish *Solenopsis invicta* from *Solenopsis xyloni*?

2:00 – 2:20 K. McCubbin, C. Jennings, Overview of the eradication efforts for red imported fire ant in Australia

2:20 – 2:40 C. Russell, RIFA pest detection efforts in Hawaii and the Pacific

2:40 – 3:40 Break (Posters attended for discussion)

Session: Behavior & Chemical Ecology

Moderator: Shannon James

3:40 – 3:55 M. Keck, **S. B. Vinson**, R. Gold, Invasive interactions of *Monomorium minimum* and *Solenopsis invicta* infected with *Thelohania solenopsae*

3:55 – 4:10 R. Renthal, S. Younger, D. Velasquez, A. Cassill, Fire ant odor and pheromone reception: A role for lipoproteins?

4:10 – 5:10 C. Russell, Video "The ant that ate America" The largest ant in the world. **PRIZE DRAWING**

6:30 – 10:00 pm Dinner at the hotel
(Beach View Room - provided)

Thursday, March 24

7:30 – 8:15 am Continental Breakfast

8:15 am General comments (Ron Weeks)

Session: Biology/Ecology

Moderator: Tim Davis

8:25 – 8:45 T. Davis, C. Allen, M. Horton, Co-occurrence of *Solenopsis invicta* with native ant species in South Carolina

8:45 – 9:05 A. Calixto, M. Harris, A. Knutson, C. Barr, K. Winemiller, Diversity and interactions of ant assemblages with the red imported fire ant in pecans, Robertson Co., TX

9:05 – 9:20 T. Bedford, W. Grant, S. B. Vinson, Red imported fire ant impact on native ants and litter removal in the post oak savannah of central Texas

9:20 – 9:35 T. Menzel, T. E. Nebeker, Imported fire ants in forested landscapes

9:35 – 9:50 S. Self, **T. E. Nebeker**, Christmas tree farmers' perceptions and practices associated with imported fire ants

9:50 – 10:20 30 Minute break

Moderator: David Oi

10:20 – 10:35 L. Glover, **T. Davis**, M. Horton, Exclusion of the red imported fire ant to prevent predation on the Eastern Bluebird *Sialia sialis*

10:35 – 10:50 S. Valles, R. Pereira,
Up-regulation of a transferrin gene in response to fungal infection in *Solenopsis invicta*

Session: Biological Control

10:50 – 11:05 M. W. Hale, S. B. Vinson, The host/parasite relationship between a microsporidian parasite *Thelohania solenopsae* and *Solenopsis invicta*

11:05 – 11:20 R. K. Vander Meer, C. Preston, G. Fritz, Prevalence of *Thelohania solenopsae* infected *Solenopsis invicta* newly mated queens within areas of differing social form distributions

11:20 – 11:35 V. Karpakakunjaram, A. Chan, R. Wright, Status of *Thelohania solenopsae* in the red imported fire ant population in Oklahoma: Implications from a 2-year survey

11:35 – 1:00 pm Lunch
(on your own – return by 1:00 pm)

Moderator: L. C. "Fudd" Graham

1:00 – 1:20 H. Fadamiro, L. Chen, Factors
affecting longevity of phorid fly, *Pseudacteon tricuspis*: Effects of sugar availability, temperature, mating, and size

1:20 – 1:35 L. C. Graham, V. Bertagnolli, M. Mobley, Phorids can be released in cold weather: A history of Alabama releases

1:35 – 1:50 V. Bertagnolli, L. C. Graham, Host location behavior of *Pseudacteon curvatus* in Alabama

1:50 – 2:05 P. Parkman, K. Vail, T. Rashid, J. Oliver, R. Pereira. S. Porter, M. Shires, G. Haun, S. Powell, L. Thead, J. T. Vogt, Establishment and spread of *Pseudacteon curvatus* in Tennessee

2:05 – 2:20 R. Weeks, Space to place phorid fly monitoring systems

2:20 – 2:50 30 Minute break

Session: Areawide
Moderator: Roberto Pereira

2:50 – 3:05 R. Wright, W. Smith, V. Karpakakunjaram, Summary of red imported fire ant areawide management program in Oklahoma 2001-2004

3:05 – 3:20 T. Davis, M. Horton, Release and spread of the fire ant decapitating fly *Pseudacteon curvatus* in South Carolina

3:20 – 3:40 C. Barr, A. Calixto, F. Mitchell, *Thelohania* infection and corresponding decreases in fire ant mound densities

3:40 – 4:00 R. Pereira, S. Porter, Use of baits for evaluation of fire ant populations in the USDA Areawide project

4:00 – 4:20 L. Gui, J. Seiner, D. Streett, R. Hasse, M. Matias, An image tracking technique for quantifying motion of black imported fire ants on a planar surface

Moderator: Brad Vinson

4:20 – 4:45 Question/answer period with International visitors, China and Taiwan

Moderator: Ron Weeks

4:45 – 5:00 Wrap up – next meeting site

POSTERS

Presenters available at posters Wed. from 2:40 - 3:40

Behavior/Chemical Ecology

• Correlation of colony social behavior and infection with *Thelohania solenopsae* (Phylum Microspora) in red imported fire ants in Texas. E. Ashbaugh, K. Logan, F. Mitchell, **S. B. Vinson**, K. Snowden

• Defensiveness of the fire ant, *Solenopsis invicta*, increases during colony rafting. **K. Haight**

• Antimicrobial activity of Solenopsin B and Isosolenopsin B. **H. White**, D. Sullivan, G. Howell, R. deShazo, R. Rockhold, H.M.T. B. Herath, N.P.D. Nanayakkara

• Isolation and characterization of venom proteins of RIFA queens. J. HaghPour, R. Renthall, **R. Deslippe**

• Cytochrome P450 gene expression in the red imported fire ant, *Solenopsis invicta* Buren. **N. Liu**, L. Zhang, H. Wang

Molecular Biology

• Comparison of glutathione *S*-transferase activity from the red imported fire ant, *Solenopsis invicta*, and Argentine ant, *Linepithema humile* (Mayr). **Z. Jiang**, L. Hooper-Bui, J. Ottea

• Molecular identification of the red imported fire ant *Solenopsis invicta* imported into the Chinese mainland. J. Yuan, **H. Pang**, H. Zhou, G. Liang, Y. Wang, Y. Zhang, J. Li, Y. Pang, L. Qu

Regulatory/Extension/Survey

• Real-time detector for fire ant mounds in pastures. F. To, **J. Reed**, D. Smith

• Development of a quarantine survey bait attractant for the red, hybrid, and black imported fire ants. **R. Jones**, A. Callcott, T. Lockley

• Red imported fire ant, a seriously harmful potential invasive species. **N. Reimer**, C. Okada

Areawide

• Impact of *Solenopsis invicta* on arthropod diversity at three sites in southeastern Oklahoma. **V. Karpakakunjaram**, R. Grantham, R. Wright

• Effect of digging behavior on efficacy of fipronil against red imported fire ants: Result of a laboratory study. **J. Chen**

• An improved method for fast fire ant colony separation. **J. Chen**, X. Wei

• Area wide suppression of fire ants: Demonstration project in Mississippi, 2004. **D. Streett**, A. Pranschke, A. Callcott

Biological Control

• Spread of *Thelohania solenopsae* in black/hybrid fire ant populations in Mississippi: Failure or success. **D. Streett**, A. Pranschke

• Survey of bacteria and fungi associated with red imported fire ants *Solenopsis invicta* Buren: An effort to discover potential microbial control agents. **S. Woolfolk**, R. Baird, D. Goodman

• Isolation and molecular characterization of bacteria from the red imported fire ant (*Solenopsis invicta*) midgut. **H. Li**, F. Medina, S. B. Vinson, C. Coates

• Genetic engineering of midgut bacteria from red imported fire ant (*Solenopsis invicta*). **H. Li**, F. Medina, C. Coates, S. B. Vinson

• Range expansion of two phorid flies in Alabama. **L. C. Graham**, V. Bertagnolli

• Host range of the microsporidium pathogen *Varimorpha invictae* at three field sites in Argentina. **S. Porter**, S. Valles, T. Davis, J. Briano, L. Calcaterra, D. Oi, A. Jenkins

• Revision of the South American thief ants (Hymenoptera: Formicidae: *Solenopsis*): A potential biological control agent for the red imported fire ant. **J. Pacheco**, W. Mackay, E. Walsh, P. Vargas, I. Del Toro, I. Moreno

Biology/Ecology

• Distibution of imported fire ant populations in Alabama. **L. C. Graham**, R. Vander Meer, K. Ward, R. Ward, V. Bertagnolli

• The effect on native ant biodiversity in northern Mississippi by the black imported fire ant (*Solenopsis richteri*) Forel. **J. Marquess**, J. Anderson

• Modeling the distribution of ants in South Carolina: Co-occurrence of the red imported fire ant and native ants in South Carolina. **T. Davis**, C. Allen, M. Horton

• Red imported fire ant colony diagnostics using worker head width. **D. Colby**, L. Inmon, L. Foil

• Affect of solar insolation on the distribution of the red imported fire ant. **A. Hernando**, J. Bush, J. Walton, G. Keller, M. Ortiz, J. Gardea, W. Mackay

• The imported fire ant mound in three dimensions. **J. Vogt**, S. Coy

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