Conference Proceedings

2010
IMPORTED FIRE ANT
AND INVASIVE ANT
CONFERENCE

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April 19-22, 2010

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Proceedings of the 2010 Imported Fire Ant and Invasive Ant Conference
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Proceedings Disclaimers

These proceedings were compiled from author submissions of their presentations at the 2010 Imported Fire Ant and Invasive Ant Conference, held on April 19-22, 2010 at the Doubletree Little Rock Hotel in Little Rock Arkansas. The 2010 annual conference was organized by the University of Arkansas, Division of Agriculture, Department of Entomology located in Fayetteville, Arkansas.

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http://www.extension.org/pages/Proceedings_of_the_Imported_Fire_Ant_Conference
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Remarks from the Conference Chairman

Greeting from Arkansas to fire ant and invasive ant workers,

I want to say a big “THANK YOU” for attending the 2010 conference held at the DoubleTree Hotel in downtown Little Rock. I hope you all had a great time and returned home enlightened with new imported fire ant and Rasberry crazy ant knowledge. For those wondering, our final tally of attendees was 92.

This conference was kicked off with a welcome from Rob Wiedenmann, our Entomology Department Head, followed by a Rasberry crazy ant session led by Bart Drees and David Oi. On the second day of our conference, Steve Yanoviak provided an informative and entertaining keynote presentation on the “Behavior and Ecology of Ants in the Tropical Forest Canopy”. Although his topic was not on fire ants, it was VERY well received. In all, 25 oral papers and 16 posters on topics pertaining to management, biology/ecology, biological control, regulatory issues and extension were presented. The final presentation of the conference was a panel discussion on “Gaps in Knowledge in Research and Extension in Fire Ants” led by Linda Hooper-Bui. Also several extension participants stayed an additional day to participate in the eXtension workday led by Kathy Flanders.

The 2010 conference would not have been possible with the dedication and hard work of our conference committee. I must acknowledge the dedicated assistance of staff from the University of Arkansas, Division of Agriculture, Department of Entomology in preparation of and during the conference, and with the proceedings publication: specifically, John Hopkins for developing the program, meal planning and this proceedings; Shelby Goucher for registration, purchasing, and accounting; Ricky Corder for registration, meal planning and program printing; and Rob Wiedenmann for advice, registration and welcome address.

Finally, we must all, conference organizers and participants, thank our industry sponsors for making the conference financially possible.

I hope to see you all at next year’s conference in Texas. Please be on the lookout for emails with details on the 2011 Imported Fire Ant Conference.

Kelly Loftin
Chairman, 2010 Imported Fire Ant and Invasive Ant Conference
Acknowledgements

The organizers of the 2010 Imported Fire Ant and Invasive Ant Conference would like to express their gratitude to the sponsors for their generous support. Their support was essential in making this conference a success. Their ads can be found at the end of the proceedings.

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We would like to thank Melanie Gould and Kelly Fleming and staff of the Doubletree Little Rock Hotel for the excellent accommodations and service in meeting the needs of the conference participants and organizers. We also thank Dennis DeVoto from Conference Direct for his assistance in hotel selection and negotiations.

We must also thank Steve Yanoviak, University of Arkansas at Little Rock for his informative and entertaining keynote presentation “Behavior and Ecology of Ants in the Tropical Forest Canopy”.

A special thanks to Jon Zawislak for his artistic talent in preparing the graphics used in conference publications and on the t-shirts.

Thanks also go to former conference organizers, Fudd Graham and Kathy Flanders for their most helpful advice.
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DSP13) Bait Insecticides and Hot Water Drenches Against the Little Fire Ant, Wasmannia auropunctata (Roger) (Hymenoptera: Formicidae) Infesting Containerized Nursery Plants. Arnold H. Hara, Christopher M. Jacobsen, Kyle Onuma

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Abstracts/Presentations

Applied Research Update: Rasinberry Crazy Ant, *Nylanderia* sp. nr. *Pubens*

Bastiaan “Bart” M. Drees, Danny McDonald, Paul Nester, Alejandro Calixto, Corrie Bowen and Roger Gold

I. Outreach Education Efforts: [http://UrbanEntomology.tamu.edu](http://UrbanEntomology.tamu.edu) and [http://AgriLifeBookstore.org](http://AgriLifeBookstore.org)
- Marketing leaflet, Public Service Advertisement (PSA)
- TPWD-AgriLife Leaflet
- Control of Rasinberry Crazy Ants In and Around Homes and Structures - Bastiaan M. Drees, Paul Nester, and Roger Gold

II. Species? Rasinberry crazy ant: *Nylanderia = Paratrechina* sp. nr. *pubens* (LaPolla, J. S., S. G. Brady and S. O. Shattuck. 2010, Systematic Entomol. 35: 118-131)
- Alate male and female forms observed Nov. 2009 (Calixto and McDonald)

III. History and Spread
- Discovered in 2002 and spread to 14 Texas counties
- Spreading by ground and through movement of infested articles

IV. Agricultural Impact
- Spread in Infested Articles: Movement of Queen(s) and Brood
- Dooryard plants, nursery trays, mulch, yard waste, trash, etc.

V. East Columbia, Brazoria Co.: Neighborhood treatment program using Advance® Carpenter Ant Bait (abamectin), BASF
- Advance® is labeled at a 1 lb/acre rate and can only be used for residential areas
  - With cooperating residents of East Columbia, TX, 3 treatments were made throughout 2009 (May 6<sup>th</sup>, May 28<sup>th</sup> and Sept. 18<sup>th</sup>
  - 59% control 6 days and 30% control 13 days post treatment in neighborhood property compared to untreated control area

VI. Other experiments with Advance® Carpenter Ant Bait
- 1 vs. 3 lb/a trial, East Columbia, TX, (May 2009); Mean No. ants in Disturbed and Undisturbed Dusted Cup Samples
- Food Lure Preference Testing in Laboratory and Field ((Jan. 6, 2009, East Columbia, Brazoria Co., TX); (Mean no. ants on each food lure over time, Jan. 5, 2009)
- Rasinberry Crazy Ant Bait Product Preferences; (May 6, 2009, East Columbia, Brazoria Co. TX)

VI. Hydrolyzed Fish Protein Modified Extinguish® Plus Ant Bait Central Life Sciences

VII. Conclusions
- Rasinberry crazy ants are not attracted to most red imported fire ant bait products
  - Advance® Carpenter Ant Bait is the most attractive to both ants, but the active ingredient, abamectin, or rate is insufficient to provide acceptable levels of control of Rasinberry crazy ants
  - Modification of Extinguish® Plus (hydramethylnon plus methoprene) shows promise in making ant bait products more attractive to these ants
Abstract - *Nylanderia pubens* in Florida: Seasonal Population Trends, Distribution and Observations

Dawn Calibeo-Hayes and Faith Oi

In 2005, an infestation of Caribbean crazy ants, believed to be introduced via landscaping materials, was discovered at the Jacksonville Zoo (Jacksonville, Florida). Two years post-introduction, the ants infest over 140 acres of the zoo and are a concern to patrons who often mistake them for red imported fire ants. In addition, these ants infest animal feed and forage, invade buildings becoming a nuisance for occupants, nest in electrical switch boxes causing shorts and service interruptions and have even brought the zoo’s train to a halt due to the density of trailing ants on the tracks.

As a first step toward developing an Integrated Pest Management program for the zoo, the baseline population and distribution of the ants at the zoo was mapped. Maps were created by placing slices of Vienna sausage at 136 preselected waypoints marked by GPS in twelve separate sampling sites at the zoo. After 5 minutes the numbers of ants foraging on the bait were counted and recorded. The bait counts were conducted approximately every six weeks from June 2008 – June 2009. Weather data were also recorded.

From the study start, the highest numbers of ants foraging on baits were found at sampling sites 1, 2, 3, 11 and 12. Although there were fluctuations due to weather, the counts remained consistent through the length of the study. Most of the heavily infested sampling sites are on the perimeter of the zoo, adjacent to wooded natural areas with ideal nesting habitat and because of the distance from the main areas of the zoo and considerable acreage, are unlikely to be treated with insecticides. Sampling site 1 is the pumping station for the Zoo’s Stingray Lagoon. Ants were introduced to this area in pallets of salt that were transported from another heavily infested area of the zoo. The salt pallets, pumping equipment and surrounding natural area of heavy vegetation provide ideal harborage for this tramp ant. Sampling site 11 is most likely the epicenter of the original infestation. This area receives and stores landscape materials for new exhibits and seasonal landscaping projects. Throughout the study, the heaviest infestation of ants has been at this site. Even when extremely cold weather reduced the population of ants in other areas of the zoo, ants in sampling site 11 survived by nesting under the black landscaping fabric.

Overall, the relative number of ants at each sampling site remained consistent compared to the other sites. This suggests that the Caribbean crazy ant is not migrating too far from the points of introduction. It is most probable that the spread of the ant throughout the zoo has been expedited by transportation of infested materials.

Recorded data also suggest that weather may have a negative impact on the Caribbean crazy ant population. Fewer ants foraged on baits during periods of cold weather and extremely cold weather can have an effect for several months. Ant counts following several consecutive nights of temperatures below 0°C were fewer than at similar average temperatures the previous year. Also fewer ants were found foraging while raining and in the few weeks following an extreme weather event.
Overall conclusions are that ant movement at the zoo appears to be mainly by transport from human activities and average temperatures below 60°F, short periods of temperatures below 32°F and heavy rain events have a significant impact on ant foraging (populations).
Introduction. The dawn of Rasberry crazy ants (Nylandaria sp. nr. pubens) has brought new attention to ant taxonomy and how we cope with invasive and potentially new species. Discovered in 2002 by pest management professional Tom Rasberry, these ants have cut a path across Texas and have now yielded reported sightings in Louisiana. It has been recently reported in Mississippi (MacGown and Layton 2009). We discovered supercolonies of crazy ants in Port Allen, LA in September 2010. James Trager recently confirmed that those ants are Nylanderia sp. nr. fulva. Although these two populations of crazy ants are being referred to by different names, the problem of ants that form supercolonies is the same. Until the taxonomy is revised by John LaPolla, the population in Port Allen, LA will be referred to as crazy ants that form supercolonies.

Background Information on N. nr. sp. pubens. This reddish-brown ant is seen in disorganized foraging trails that enter houses and trees. Legs and antennae are usually longer than the body, which is characterized with coarse hairs. They have a one-segmented petiole and 12-segmented antennae. They do not possess a sting but can spray formic acid. They are best identified by examining the color and number of macro-chaetae. These ants are known to be omnivorous and will feed on items such as dead insects and honeydew. Their colonies contain numerous queens, are unicolonial, and are not known to extensively excavate soil. Little is known about their natural history; however early evidence suggests that they can be compared to the Argentine ant (Linepithema humile), yellow crazy ant (Anoplolepsis gracillipes), and the European garden ant (Lasius neglectus) which LaPolla (2010) used as an outgroup for taxonomic studies (See Table 1).

Table 1. Comparisons between crazy ants that form supercolonies and European garden ant, Argentine ant, yellow crazy ant, and fire Ant.

<table>
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<tr>
<th></th>
<th>Crazy ants</th>
<th>EGA</th>
<th>AA</th>
<th>YCA</th>
<th>Fire Ant</th>
</tr>
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<tr>
<td>Low Temp Activity</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Colony Structure</td>
<td>Unicolonial</td>
<td>Unicolonial</td>
<td>Unicolonial</td>
<td>Unicolonial</td>
<td>Monodom./Polydom.</td>
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<tr>
<td>Mode of Distribution</td>
<td>Fission</td>
<td>Fission</td>
<td>Fission</td>
<td>Fission</td>
<td>Mating flight and fission</td>
</tr>
<tr>
<td>Impact on Local Fauna</td>
<td>Large</td>
<td>Large</td>
<td>Large</td>
<td>Large</td>
<td>Large/min.</td>
</tr>
<tr>
<td>No. Queens</td>
<td>Hundreds</td>
<td>Hundreds</td>
<td>Hundreds</td>
<td>Hundreds</td>
<td>Many 10s</td>
</tr>
<tr>
<td>Mating</td>
<td>Internidal?</td>
<td>Internidal</td>
<td>Internidal</td>
<td>Internidal/ flights rare</td>
<td>Flights</td>
</tr>
<tr>
<td>Nest Structure</td>
<td>Shallow/little excavation</td>
<td>Shallow/little excavation</td>
<td>Shallow/little excavation</td>
<td>Shallow/little excavation</td>
<td>Extensive</td>
</tr>
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Meyers and Gold (2008a) first studied this ant and alerted researchers to the high populations that are seen throughout the invaded areas of Texas. They present anecdotal evidence that Raspberry crazy ants homogenize other ant fauna. Meyers’ and Gold’s (2008a) morphometric analysis demonstrates differences in morphology. They put specific focus on the number of macrochetae that provides evidence that the ants may be different from described species of crazy ants. They offer additional evidence that this ant is a new species by noting differences in behavior from other ants such as the Caribbean crazy ant, Nylandaria pubens such as biting. Although federal authorities are aware of the growing concern about crazy ants that form supercolonies, at this present time no federal expansion prevention has been made (Meyers 2008, Meyers and Gold 2008a, C. Brown pers. comm.). Meyers and Gold (2008b) also tested two toxicants as bait in the laboratory.

Management Plan for Crazy Ants that Form Supercolonies. Area-wide or community-wide management of fire ants and Argentine ants has been successful in Louisiana for more than nine years. The information presented here is based on modified techniques that were developed for Argentine ant management due to the ants’ similar biology. These techniques have not been fully tested on crazy ants that form supercolonies. They are based on preliminary results and strong results we have from Argentine ants. Argentine ants have very similar biology and the following recommendations are the result of five years of our research. The following protocol must be done with most people in the area at the same time in order to be successful. It must be stressed that the process must be repeated after six weeks and possibly after 12 weeks. This recommendation is based on the knowledge we have about the natural history of Argentine ants and other polygynous ants; they have queens that are rarely fed and are kept in reserve that will not be fed bait until the later treatments. We suggest that early April is the best time for this protocol to be implemented, but this can be done at any time. However, it will be more successful if the crazy ant populations are disrupted before they grow too large and unmanageable. If new infestations are observed and treated early enough, the crazy ants that form supercolonies may be eliminated and the ant and arthropod biodiversity can be preserved.

1) Monitoring and identification: Correct identification of the crazy ant is important. Send the ants to an expert who will identify them and send them to the North American experts on Nylanderia for verification. Once the ants have been identified as crazy ants that form supercolonies, the following integrated pest management (IPM) protocol should be followed.

2) Sanitation: Clean up the area. Remove harborage that provide nest sites such as fallen logs, children’s toys, overturned boats, pots with soil, free-standing basketball nets, woodpiles, lawn tools and other items that crazy ants can nest in and under. These items also provide extra heat for rapid development of colonies, which can quickly overwhelm the area. Trim plants and trees so that they do not touch the structures. Ants can gain entry into structures from tree branches that are simply close to the roof. Crazy ants are very attracted to spaces that are moist and wood that is rotting. Rotting wood provides moisture and heat needed for colony reproduction. To prevent infestation, consider replacing rotting siding, window sills, and door frames on homes and outbuildings. Look for ground-to-wood contact around structures and find a way to eliminate this problem. The next time it rains, walk around the property and look for water that may pool
or contact the wood on the structures. Eliminating water-soaked wood will help prevent ant infestation of homes and siding.

3) **Disrupt the foraging into trees and structures:** Using a contact insecticide in a handheld pump or backpack sprayer, apply a liquid barrier around trees approximately two feet up and one foot out the base of the tree on which the ants are trailing and around houses or other structures that are infested. Make sure to treat all surfaces of the bark of the trees and try to treat all the trees on your property at the same time. If possible, drench with contact insecticides any nests of crazy ants seen. This will partially disrupt the nesting and foraging and allow the rest of the treatment to be successful.

4) **Destroy visible nests:** Using the same technique described above, use a contact insecticide to destroy any visible nests. Many of the nests are not detectable by humans, so the next step is to employ the natural behavior of the ants to do the work to suppress their own nests.

5) **Let them eat bait (use small particles!):** This process capitalizes on the ants amazing foraging behavior to gather a palatable bait that contains a small amount of insecticide. Broadcast a granular bait in the early spring. Colleagues in Texas report that the Rasberry crazy ants are attracted to Whitmire Advance Carpenter Ant Bait but have had limited success when one broadcast application is conducted. It is important that you use fresh bait and apply it when the ground is dry and no rain is expected for 24 hours. Broadcast bait over the entire infested area. Liquid baits can be tested for palatability and offered in bait stations. Hooper-Bùi’s experience with other species of crazy ants indicates that liquid baits can be effective. Ants will usually visit liquid bait stations when they need sugars or carbohydrates and may come to them intermittently. Be sure to place the bait stations out of direct sunlight; ants will not enter a bait station that is hot.

6) **Repeat after six weeks and again after 12 weeks.** Repeating this process is important as this polygynous ant species potentially has reserve queens.

**Survey.** In 2009, our laboratory began an extensive statewide survey to determine both potential presence of Rasberry crazy ants and which ant species were still viable in post-hurricane areas. We sent packages containing vials, checklists, and instructions on how to collect ants for research to 65 county agents and pest management professionals. Unfortunately, this process has been very slow in that weather conditions have impeded results for the survey. Members of this laboratory still conduct opportunistic collection whenever possible. In 2010, we sent packages to pest management professionals across the state in hopes of furthering our survey. Few of these boxes have been returned.

At this present time, several unconfirmed sightings of crazy ants that form supercolonnies have been reported. These areas include St. Landry Parish, Jackson Parish, Port Allen, Calcasieu, Shriver, New Orleans, Johnson’s Bayou, Morgan City, Slidell, the Lakeshore area, and the state of Mississippi (Joe MacGown). Extensive searching has been conducted in the city of Port Allen. Hooper-Bùi and Chen have discovered over 14 species of ants in areas adjacent to the city’s initial infestation. One population has caused particular concern in Port Allen. The large infestation includes a home, a farm, a pipeline construction area, and the parish (county) public
works yard. Observations have yielded discovery of Louisiana crazy ants patterning foraging activity after EGA. The ants are active at lower temperatures (52 F) than is typical for other ants in the south. Extended freezes (>36 hours) severely impacted the population but did not eliminate them.

**Need for USDA to be Actively Involved.** Another major area of concern is the presence of ants in the Port Allen Department of Public Works Yard. The ant has infested sand and other materials that are routinely transported around the parish (county). It has also infested a pasture used for hay production (infested hay is shipped outside the parish), and area homes and business (tourist information building and truck stops. We have reported the findings to USDA-APHIS and Louisiana Department of Agriculture and Fisheries. Provided that funding can be secured, we can document the effects of these invasive, unicolonial ants on the natural ant fauna in natural, urban and agricultural areas. If we mobilize quickly, we have the opportunity to conduct research to quantify the ecological effect of this invasive ant species. This species will radically impact urban and agricultural systems.

For example, ecological meltdown caused by yellow crazy ants (*Anoplolepsis gracilipes*) on Christmas Island has been eloquently researched (O’Dowd et al. 2003). However, their research began about 10 years after the ant invaded the area and the damage they caused to the ecosystem had to be inferred from studying and comparing invaded and non-invaded areas. Lach and Hooper-Bùi (2009) state whereas most studies on the effects of invasive ants document suppression of native species, few studies take an experimental approach, and that most studies have been conducted with *Solenopsis invicta* or *Linepithema humile*. D. Gordon, her colleagues, and students worked in the Jasper Ridge Biological Preserve where they document Argentine ant (*L. humile*) populations were encroaching on a natural area (Sanders et al. 2003). Another method for examining the effects of invasive ants on native species is to conduct removal experiments or introduction experiments (King and Tschinkel 2008) that cause massive controversy among invasion ant ecologists.

We have an opportunity to examine the invasion biology of an emerging, invasive species that no one has been able to study in this way. This opportunity is unique in that we can study the effects of this native ant on the natural ant population in urban, agricultural and natural areas. The research must be initiated now, before the weather warms in the spring and the colonies of this invasive ant begin expansion.
Overview of USDA Imported Fire Ant Program

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Imported Fire Ants in the United States

Black Imported Fire Ant Solenopsis richteri

Red Imported Fire Ant Solenopsis invicta

Hybrid imported Fire Ant Solenopsis invicta x richteri

Potential Range of the Red Imported Fire Ant

Spending $1.9 million to keep IFA where they are
Saves nursery growers where they could be $3.6 million
Saves homeowners where they could be billions of $
**History**

Cooperative eradication program expands

1965-1977

Mirex bait developed

**History**

Converted WWII aircraft used to apply mirex bait. 1962-1978. More than 140 million acres treated

**History**

Shift from eradication to management

1978-2000

**History**

Quarantine funding restored in FY2000. FY 2010 = $1.9 m

**Goal of Present-day APHIS IFA Quarantine**

Prevent the artificial spread of imported fire ants
**Movement of Nursery Stock**

- Containerized Nursery Stock
  - Pre-plant granular incorporation of insecticide
  - Drench with liquid insecticide

- Field-grown or balled-and-burlapped Stock
  - Broadcast bait + broadcast contact insecticide for field-grown stock
  - Drench or dip/mixes for balled stock

**Grass Sod**

- Broadcast applications

**Bees and Equipment**

- Bee equipment was implicated in the original CA IFA infestation

- Since then, apiary equipment has been rigorously inspected in the western states

**Baled Hay and Straw**

- Baled hay and straw are regulated items. Currently, these items are ineligible for movement if stored in direct contact with the ground

**Implementation of Quarantine**

- Since the late 1980’s, the federal IFA quarantine program has been implemented by the states

- States are responsible for
  - Inspecting nurseries
  - Issuing compliance agreements
  - Conducting blizzards with USDA
  - Survey

- Oversight by the USDA
  - Development of quarantine treatments
  - Transfer of information to states
  - Enforcement: investigations and fines associated with violations

**Federal Imported Fire Ant Quarantine**

- Partially or fully quarantined states personnel

- USDA-APHIS-FBQ

- State Personnel

- Federal
  - IPM Program Manager
  - Field Health Directors

- Eastern Region
  - IPM Program Manager
  - State Directors

- Western Region
  - IPM Program Manager
  - State Directors

- State Inspectors
  - Regulatory Officers
  - State Inspectors
Compliance Agreements

- Agreement between a nursery or grower and USDA-APHIS (issued through the state) whereby the grower agrees to treat his nursery stock or material in a specified manner prior to shipment outside the IFA regulated area.
- Grower allows a stamp or certificate to all treated stock/materials or invoices for all materials shipped outside the IFA regulated area.
- State personnel review a new compliance agreement on a yearly basis.
- State personnel watch 1-2 treatments per year to insure compliance.
- State personnel can request treatment records from growers.

Survey

Primarily used in the U.S. to find IFA colonies in isolated infestations (areas where IFA do not normally occur) – could be used for survey of port areas.
- Use bait attracting traps to detect IFA colonies in small areas.
- Place traps when the temps are 60°F-82°F (16°C-28°C).
- Leave traps in place about 1 hour and collect.
- Areas > 10 acres (4 ha):
  - Bait areas with visible mounds
  - Place traps every 200 feet (60 m) in grid pattern
- Areas < 10 acres (4 ha):
  - Areas with no to few visible mounds
  - Place traps every 50 feet (15 m) in grid pattern
- Trapping patterns may be more intense as situations dictate.

Weigh Station Blitzes

- Blitzes are "surprise" inspections of trucks at weigh stations on the U.S. highway system.
- State and federal personnel man these blitzes.
- Paper work is inspected.
- Cargo is inspected.
- Soil samples are taken.
- If ants are found:
  - Shipment placed on hold
  - Ants identified
  - Shipment:
    - Resealed to origin
    - Destroyed
    - Treated.

Residue Analysis for Compliance

- Routine soil samples collected annually by state inspectors at nurseries under compliance agreements.
- Soil samples collected during blitzes.
- Residue analysis either by USDA lab or state lab.
- Results from USDA lab 2007-2009:
  - Routine samples – 52% contain program insecticides.
  - Blitz samples – 75% contain program insecticides.

Website

USDA-APHIS-PPQ Imported Fire Ant site

Thank you

Charles Brown, Anne-Maria Caluori, Stacy Scott, Tom Weeks
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Myth Busting with Social Media

Putting New Tools to Work in Extension

Maggie Lemoine
Communications and Marketing Specialist
Alabama Cooperative Extension System

What Do Extension Professionals Do?
- Talk with clients
- Listen to problems and concerns
- Help find solutions
- Collaborate with colleagues and others

What Does Social Media Do?
- Develops conversations
- Provides opportunities to listen
- Creates means to publicize solutions
- Provides another tool for collaboration

Social Media Just Another Set of Tools
- Examples of popular social media outlets

Are we where the people are?
- 27% of U.S. have heard of Cooperative Extension.

People are here:

<table>
<thead>
<tr>
<th>Social Media</th>
<th>Users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facebook</td>
<td>400 million</td>
</tr>
<tr>
<td>MySpace</td>
<td>175 million</td>
</tr>
<tr>
<td>Twitter</td>
<td>75 million</td>
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<tr>
<td>LinkedIn</td>
<td>60 million</td>
</tr>
<tr>
<td>YouTube</td>
<td>96 million videos (March 2009)</td>
</tr>
</tbody>
</table>
Old way

One to one (no scaling).
Broadcast (one to many).
Controlled publishing.
Static.

New Way

Interactive
Relevant
Scalable
Inclusive
Responsive
Timely

48% of Americans have a Facebook or MySpace account.
(April 2009)

New way allows

Fast connections
Easy publishing
Collaboration
Going where the people are
Maintaining relationships with clients
Building & confirming reputations online

Answer "What is happening?"
Share thoughts.
Share links.
Ask questions.
Answer questions.
...in 140 characters or less.
Learn to look at online differently

Think of online social circles much like we think of face-to-face social circles. Photo: www.flickr.com/photos/carface/2446138697

Transparency is whether you are online or not

Photo: flickr.com/photos/bsrsc/5453949790

Listen
Learn
Connect
Share

Photo: www.flickr.com/photos/alpemy/184573859

Many thanks to Dr. Anne Adrian for the generous use of many of her slides for this presentation.
Spread of fire ant decapitating flies \textit{Pseudacteon tricuspis} and \textit{Pseudacteon curvatus} in Louisiana

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Introduction

The decapitating flies \textit{Pseudacteon tricuspis} Borgmeier and \textit{P. curvatus} Borgmeier (Diptera: Phoridae) are small South American endoparasitoids of worker ants in the genus \textit{Solenopsis}. Phorid parasitoids were successfully released in the United States beginning in 1997 (\textit{P. tricuspis}) and 2000 (\textit{P. curvatus}) (Callcott \textit{et al.} 2009) as classical biological control agents of the imported fire ants \textit{Solenopsis invicta} (Buren), \textit{Solenopsis Richteri} Forel, and their hybrid. In cooperation with the USDA-ARS and the USDA-APHIS, Louisiana State University AgCenter successfully released \textit{P. tricuspis} (4 sites) and \textit{P. curvatus} (3 sites) in Louisiana (Callcott \textit{et al.} 2009). In 2008, \textit{P. tricuspis} occupied about 706,000 km² (50% of the fire ant quarantine area) eleven years after its first release in the United States whereas \textit{P. curvatus} occupied 825,000 km² (60% of the fire ant quarantine area) eight years after its first release (Callcott \textit{et al.} 2009). The objective of this study was to evaluate the status of these fire ant parasitic flies in Louisiana in 2009.

Materials and Methods

In total, 136 sites located on roadsides bordered by forests, agricultural fields or pastures were sampled (Figure 1). At each sampling site, 10 fire ant mounds were selected and sampled. Sampling was conducted between 1130 and 1800 h and when ambient temperatures were warm enough for phorid fly activity (>20°C). Mounds were disturbed and ants were crushed to provoke the release of their alarm pheromone. Each mound was observed for 2-3 minutes. All flies were collected with an aspirator and brought to the laboratory for identification. Identified flies were sexed (\textit{P. tricuspis}) and counted.

Results

\textit{P. tricuspis} currently (2009) occupies 46 out of 64 parishes in Louisiana, approximately 86,900 km². \textit{P. tricuspis} has not been collected in the northern and middle parts of the state. The greatest number of flies (262/10 mounds), including males and females, was collected in Natchitoches Parish on October 6\textsuperscript{th} between 1315 and 1345 h (Figure 2).

\textit{P. curvatus} currently (2009) occupies 57 out of 64 parishes in Louisiana, approximately 105,200 km². \textit{P. curvatus} has not been collected in the middle part of the state and in St. James and Assumption parishes. The greatest number of flies (444/10 mounds) was collected in Union parish on October 10\textsuperscript{th} between 1545 and 1615 h (Figure 3).
References


Figure 1. Phorid sampling locations in Louisiana. Locations were recorded with a GPS (Garmin etrex LEGEND HCx) and presented on LA map using Arc GIS. ( Red: Sampling location for Phorid flies; Blue: Sampling location for Phorid flies: no fire ants were found (06/20/2009)).
Figure 2. Distribution of *P. tricuspis* in Louisiana, 2009. The average number of flies per 10 disturbed mounds was calculated for each parish and broken down into 5 categories represented on the LA map using Arc GIS. The successful release sites and date of releases are also reported:
1. St. Tammany parish (1999);
2. East Feliciana parish (2000);
3. Natchitoches parish (2005);

Figure 3. Distribution of *P. curvatus* in Louisiana, 2009. The average number of flies per 10 disturbed mounds was calculated for each parish and broken down into 5 categories represented on the LA map using Arc GIS. The successful release sites and date of releases are also reported:
1. Madison (2005);
2. East Feliciana parish (2006);
Fire ant Baits and Biocontrol with Pathogens Update

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Summary

The insect growth regulator (IGR) methoprene (isopropyl-(2E,4E,7R,S)-11-methoxy-3,7,11-trimethylldodeca-2,4-dienoate) has been shown to have deleterious effects on red imported fire ants, *Solenopsis invicta*. It interferes with normal development of worker caste brood and reduces queen egg production (Vinson and Robeau 1974). Methoprene is a racemic mixture of two enantiomers (R and S in a ratio of 1:1) with the juvenile hormone activity restricted to the S enantiomer (Henrick et al. 1978, World Health Organization 2002). The S enantiomer is the active ingredient in some commercially available fire ant baits. The efficacy of fire ant bait containing only the S enantiomer (0.5% (S)-methoprene) was compared to baits comprised of a racemic mixture (0.5% R and S enantiomers in a 1:1 ratio) and a standard IGR bait containing pyriproxyfen (0.5%). Efficacy was based on worker brood reduction in laboratory colonies of *S. invicta* containing approximately 21,000 workers, 20 ml brood, and 1 queen for 6 replicates per treatment. There were no significant differences between the S and RS methoprene baits with over 88% brood reduction by the 5th week after bait access. Colonies accessing the pyriproxyfen bait had faster brood reductions; 74% at 3 weeks and 97% at 5 weeks. Colonies fed control bait of 30% soybean oil on corn grit grew in size until the 5th week when where there was 17% reduction. Symptoms of a virus infection, possibly *Solenopsis invicta* virus 3 (Valles and Hashimoto 2009), was evident at week 6 with control colonies exhibiting about 70% brood reduction. Note that this laboratory study optimized bait efficacy by providing unrestricted access to bait after colonies were starved, and may not reflect differences in field efficacy or in bait formulation.

Fire ant decapitating phorid flies can acquire the fire ant pathogen *Kneallhazia solenopsae* when they develop in infected *S. invicta* (Oi et al. 2009). To determine the prevalence of *K. solenopsae* in field-collected phorid flies, *Pseudacteon curvatus* females were collected at four sites having 0 to 64% of the *S. invicta* nests infected with *K. solenopsae*. Based on PCR detections in individual flies, infection prevalence averaged 11.5% (range, 4.8-15.6%). *K. solenopsae* infection prevalence in flies was independent of *K. solenopsae* infection in *S. invicta* nests at the collection sites (Valles et al. 2009). Preliminary studies also were conducted to determine if infected phorid flies can vector *K. solenopsae*. Infected phorid flies were confined with small *S. invicta* colonies which allowed flies to oviposit on worker ants, or to be eaten by the ants. Thus far, there has been no evidence of transmission.

The host range of *K. solenopsae* was previously thought to be restricted to fire ants in the *Solenopsis saevissima* species group. An unpublished report by Snowden and Vinson (2006) indicated natural infections occur in the tropical fire ant, *Solenopsis geminata*, in contrast to laboratory inoculations and field surveys of *S. geminata* in Florida (Oi and Valles 2009). Further examinations of samples from Mexico and Texas have detected *K. solenopsae* in *S. geminata*. 
However, preliminary phylogenetic analyses indicated possible variations in *K. solenopsae* from different hosts.

References Cited


Are the Imported Fire Ants a Detriment to some Biological Control Programs?

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Bermuda grass (*Cynodon dactylon*) is an African grass that became an important forage, lawn, and golf course grass in the early 1930’s and remains so today. In the 1940’s the Rhodesgrass mealybug (*Antonina graminis*) from China showed up (Dean *et al.* 1979). By 1960 the mealybug was considered a serious pest of several grasses (Bartlett *et al.* 1961, Chad and Wood, 1960). An effort was initiated to find an effective parasite (Riherd 1950, Schuster and Boling, 1971). One of these, the Encyrted wasp (*Neodusmetia sanguani*) from Asia, established and provided very effective control (Dean *et al.* 1979). However the parasite was and is slow to spread, as it is wingless.

In the 1980’s the Rhodesgrass mealybug began to again cause serious damage to important forage grasses. This problem began to occur in areas invaded by the Imported Fire Ant (IFA) (*Solenopsis invicta*). By early 2000 it was becoming clear that the mealybug was again becoming a serious problem and it appeared that the IFA may be playing a role (Helms and Vinson 2002, 2003), Like many ants, the IFA readily tends aphids and other Homoptera (Scarborough and Vinson 1991, Styrsky and Eubanks 2007). Thus, we set out to determine if the IFA was or is effective in protecting the mealybug from parasitism.

A study was set-up in 38 cm x 55 cm x 38 cm high plastic boxes coated with Fluon® and with a top made of fine screen. Bermuda grass was collected, cleaned and planted in 2.5 liter plastic pots and the grass was trimmed every few days to maintain a 30 cm growing length above ground. Two pots of grass were placed in each of the boxes and trimming was continued throughout the experiment. The mealybugs were collected from infested Bermuda grass and the infested stolons were trimmed, rinsed with water and then were placed in 10 ml test tubes containing a water soaked cotton ball placed in the bottom and a dry cotton plug in the top. These were then held in an incubator at 30°C. When these vials contained 50+ crawlers (in a week or so) they were then placed in the grass containing test pots (1/pot). To collect the parasites; Bermuda grass clippings with mealybugs were placed as described and were held in an incubator at 30°C for 10 days. They were then inspected daily for 22-28 days and emerging parasitoids were removed to a small cage (a plastic cup with a lid with a screen insert in the top) and the cages were provided with honey water. When 50 parasites were present they were added to the grass containing pots. Fire ant colonies were collected, removed from the soil by flotation and manipulated to form small colonies with 1 physiogastic queen, 5.5 gm of workers and 5.5 gm of brood + eggs. One fire ant colony was added to corresponding boxes 48 hrs after introducing the parasites. Boxes with fire ants were fed a half dozen crickets (frozen) and mealworms daily.

The experiments in the boxes were replicated four times and consisted of grass and mealybugs and (1) no ants and no parasites, (2) no ants but with parasites, (3) ants and parasites, (4) ants but no parasites. Some experiments were held to just prior to mealybug pupation and
others were held for two generations of the mealybugs and parasites (27 days). On evaluation of the parasitized mealybugs, they were separated into two categories (emerged - a round hole in the mealybug pupa) or (parasitized + eaten - rough edge hole in the mealybug pupae).

The results of the first generation of mealybugs that were parasitized (ants removed before parasites emerged) yielded no parasites with or without ants confirming that no parasites are produced if no parasites are introduced. When parasites were present with no ants about 30% of the mealybugs were parasitized. When ants were present about 40 % of the mealybugs were parasitized, indicating that the ants may have helped parasitism in some small way. This may be possible as ants tending the mealybugs often clean the area around the mealybug, thus increasing the ants’ access.

However, when ants were allowed to be present following pupation of the parasites and during emergence revealed that only about 2 % of the parasites emerged, the rest were consumed. If no ants were present about 35 % of the mealybugs were parasitized and emerged. The results clearly support the conclusion that the ants destroy the parasites following pupation within their host.

When parasites were present, without ants for two generations of mealybugs, 45 % of the mealybugs yielded parasites. In contrast, if ants were present only 1 % yielded parasites. We also recorded whether the mealybugs were parasitized above or below ground (first generation prior to pupation). We found that only 2 % of the mealybugs (with or without ants) were parasitized above ground while about 80 % were parasitized below ground (with or with out ants). These results confirm that the ants do not impact parasitism, but it also reveals that the parasites prefer to forage underground or in the thick grass thatch.

Overall the results confirm that the Imported Fire Ants are seriously reducing the effects of the parasite, *N. sanguani*, to provide biological control of the Rhodesgrass mealybug. This is particularly serious, as the parasite is a very poor disperser.

References:


Submission for Proceedings of the 2010 Annual Imported Fire Ant and Invasive Ant Conf.
Comparing bait mediated behavior justifications in disturbed and undisturbed fire ant colonies

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Abstract: This study investigated how a combined stimulus of nest disturbance and an immediate food offer affects task selection and relocation of nurse, forager, and reserve workers. A shift from critical task of nest defense elicited by nest disturbance to foraging at encounter of food was observed, irrespective of the role designated by their age and size. The empirical evidences attest that fire ant workers are consummate opportunists and are able of fine-tuning task allocation to fit the best interest of their colony.

Key words: red imported fire ant, behavior-flexibility, task-switching, bait, disturbing

Ants are among the most successful taxa. Their behavioral system makes them uniquely adaptable to varying environment. A classic example is the red imported fire ant (Solenopsis invicta), which was introduced into North America through Mobile, AL in the 1930s (Wilson 1951) and has since caused extensive economic and ecological damage. This is likely due in large part to their dynamic polyethism and worker caste polymorphism. Cassill and Tschinkel (1999) proposed that workers task selection is coarsely regulated by their age and size: older and large-size workers acting as foragers, younger and small-sized primarily performing brood-care, and the mid-aged and ~sized workers being the most versatile, engaging competently in food traffic and being the most defensive. Mirenda and Vinson (1981) believed that there are two main worker castes (‘nurses’ and ‘foragers’) and a large versatile ‘reserve’ subcastes. Member in each caste/subcaste span a wide age-size range. Hu and Ding (2009) observed that alerted defenders reversed to be foragers at encounter of food particles. They proposed that workers are consummate opportunists and possess behavior-flexibility, a task-selection fine-tuned by task-switching.

A behavior in insect is the product of heredity, environmental stimuli and past experiences (Wilson 1971). Behavioral tests can improve the interpretation and ecological relevance of standardized test results of pesticide toxicity and efficacy. This study was to test the hypothesis that worker castes are capable of fine-tuning task allocation to fit the best interest of their colony, though the investigation on how a combined stimulus of nest disturbance and an immediate food offer affects task selection and relocation of nurses, foragers, and reserves. a shift from critical task of nest defense elicited by nest disturbance to foraging at encounter of food, irrespective of the role designated by their age and size.

Materials and Methods

Experiments were conducted in November 2009, at mean ground temperatures of 26±1.5°C on clear days with ≤15 km/h winds. One day before the experiment was carried out, activity of fire ant mounds were identified by tapping the mound surface with a metal wire (3-
mm in diameter). Active mounds, indicated by workers responding to tapping, of similar size (diameter = 41±7 cm; height = 14±4 cm) were selected for testing.

Disturbing treatment was performed by removing the top 1/3 of the mound with a shovel. The removed part was placed along the edge of the mound. Food (a blank bait carrier) was offered immediately following the disturbance by evenly applying a tablespoon of bait around the lower 2/3 of the mound. Non-disturbed mounds were used as control. There were four replications for each of the following two experiments.

**Experiment 1**: Worker size distribution immediately following the combined stimulus of disturbance and food offer.

Ten bait particles were observed at the time they were picked up by a worker. The size types of the worker picking up and carrying a food particle were noted for a maximum period of 5 minutes.

**Experiment 2**: Sequential task selection shift induced by mound disturbance and food offer.

Four 10cm X 10cm quadrates located on the lower 2/3 of each mound were used to record changing proportions of individuals of different worker size types exhibiting aggressive or foraging behavior corresponding to the combined stimulus. Proportion estimates were made at 1-min intervals for a 20-min period, through visual observation and aided by photos taken at the designated intervals.

Workers that examined and picked up baits, removed brood, or showed no aggressive behaviors were defined as foragers; workers released aggressive behaviors (mouth opening, jetting included lunge/chase, intense antennations) were defined as defenders.

**Results**

All worker types removed bait from disturbed nests (Fig. 1a), and predominantly small workers removed bait in control nests (Fig. 1b). Bait particles were brought into the nest through exit holes or gaps generated by disturbance, and this happened over less time in disturbed nests.
In disturbed nests (Fig. 2a), proportionately more small and medium workers foraged in the first 5 min., after which medium workers foraged more. All the bait particles were moved out of where they were distributed within 1 h. In control nests (Fig. 2b), small and medium workers proportionately foraged the most until 15 minutes following bait application, after which small workers predominated. About 80% bait particles were removed in a period of 1 h. Defensive behavior was proportionately highest at 0-5 min. after disturbance and continued at reduced levels until 7 min. post-disturbance.
**Summery and discussion**

All disturbed worker types foraged once they encounter baits, despite proportionately more individuals being defensive. Effects of worker fine-tuned task allocation continued in medium workers at disturbed nests throughout the observation period. This observation indicates that accessible food items induce unplanned foraging in disturbed workers, and workers fine-tune their task allocation to foraging in disturbed nests, irrespective of the role designated by their age and size. This behavior decision could be made to balance the risk and benefit of the colony.

Control data from Experiments 1 and 2 had more small workers foraging immediately following food offer. This reflects the literature: small workers are most common within nest mounds and most likely to encounter baits on the mound surface (Haight 2008). Without major
disturbance, few defending medium workers came out and larger workers were presumably in distance from the mounds. Our study bolsters the argument that disturbing fire ant nests does not affect the ability of ants to forage poisoned bait (Hu and Ding 2009). Our results are highly significant to fire ant control within highly disturbed anthropogenic systems, such as the growing turfgrass industry.

Reference


Impact of picorna-like virus from the red imported fire ant, *Solenopsis invicta*

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Abstract

The red imported ant, *Solenopsis invicta*, was first reported in Taiwan in late 2003, and no natural enemy, especially pathogen could be detected except the picorna-like virus, *Solenopsis invicta* virus-1 (SINV-1) so far. An alternative control strategy that integrates this virus with ongoing bait treatment therefore seems feasible and may have potential to long term suppress the fire ant in Taiwan. One core issue for this strategy is that how the virus transmits within or between colonies as well as how the virus impacts its host, which remains fairly unclear given most of studies focus on its molecular characterization. Hence, the present study aims to determine the infectivity and transmissibility of SINV-1, and characterize the symptom of infected colonies. Virus particles have been purified from infected colonies (confirmed by RT-PCR) and are consistent in size and shape with those previously published under electron microscopic examination. Subsequent sequence analyses confirmed these virus particles as SINV-1 by highly similar sequence identity. However, the horizontal transmission was evident, most likely through trophallaxis from infected larvae to workers. In pathology, 4th instars larvae shows programmed cell death in epithelial cell. And artificial infected larvae displayed serial cytopathic effects through infective duration gradually. SINV-1 induces apoptosis suggests closely relation with brood death.

Key words: *Solenopsis invicta*, picorna-like virus, transmissibility

Introduction

In Taiwan, the first record of introduction of the red imported fire ant, *Solenopsis invicta*, was reported in 2003. Based on potential of the rapid establishment and proliferation, scientists suggested the fire ant will spread to whole tropical and subtropical countries in future (Morrison et al., 2004). To prevent fire ant from spreading and establishment, the large scale control has been taken place after foundation of National red imported fire ant control center in Taiwan. The fire ant control strategies advocates chemical pesticides to eliminate the infestation. In general, the fire ant control in Taiwan used delay toxic bait base on efficient transmissibility, such as Indoxacarb (Barr, 2003), Fipronil (Collins and Callcott, 1998), and Pyriproxyfen (Banks and Lofgren, 1991). According to Vander Meer et al. (2007) study, 71% control rate is reached by chemical pesticides only. However, fire ants control using integrated management that involves the combination of natural enemies (phorid fly, and microsporida) and bait, the control rate reaches 88% and significantly higher than that using chemicals only. In recently introduced areas, Australia, Taiwan and China, no enemies species was found except *Solenopsis invicta* virus-1 (SINV-1). To avoid the risk of introducing exotic bio-control agents, the alternative control strategy may consider the integration fire ant viruses with ongoing bait treatment in Taiwan. SINV-1 is a picorna like virus belonging to the genus *Aparavirus* within the family Dicistroviridae, first isolated from fire ant in 2004 (Valles et al., 2004). SINV-1 be detected all
stages of fire ant colonies in field, however with no apparent symptom. Furthermore, SINV-1 reveals brood mortality when reared in laboratory.

Given the pattern of widespread distribution of SINV-1 in Asia (Yang et al., 2010), it seems feasible to use it as a potential agent to long term suppress the fire ant in Taiwan. However, most studies of fire ant viruses emphasized the molecular characterization and viral classification of SINV-1. The core issue for integrated management is how the virus transmits within or between colonies as well as how the virus impacts its host. The object of this study is therefore to demonstrate the role of 4th larvae, as key stage for transmission of another fire ant pathogen, Knealhanzia solenops (Oi et al., 2001), for SINV-1 transmission in fire ant colonies as well as the viral impacts on 4th larva.

Material and methods

Field surveying, and virus detection

Total RNA was extracted each colonies with 10-15 fire ant workers using 0.8 ml of TRIzol reagent (Invitrogen), and eluted in 50 μl of RNase free water. And first strand cDNA synthesis was using MMLV High performance RT kit, and consisted of 20 μl of total RNA, 6 μl of 100 μM Oligo dT primer, 4 μl of 10x RT buffer, 2 μl of 0.1 M DTT, 1 μl of RT enzyme, and RNase free water to adjust the 40 μl volume. SINV-1 detected by pair primers p517/p523, the partial region of RNA-dependent-RNA-polymerase, has higher sensitivity for RNA virus detection (p517: 5’CAATAGGCACCAACGTATATAGTAGAGATTGGA, p523: 5’CCTCATTGAAGATAATCCTCTCTTGAGAAA ‘3) (Hashimoto et al., 2007). PCR program is as follows: 1 cycle at 95 °C for 10 min, 35 cycles of 94 °C for 15s, 60 °C for 15 s, 68 °C for 30 s, followed by a final elongation step of 68 °C for 5 min.

SINV-1 horizontal transmission

SINV-1 horizontal transmission route proceed through introduced 20-25 infected 4th brood and 25 healthy workers in artificial colonies. All fire ant samples were from polygyne colonies to reduce colony incompatibility, and determined whether infected brood was either killed or adopted. Colonies reared with sugar water and crickets, and five replicates detected after 14, 30 days. In experiment process, the fire ant pupae were exchanged 4th brood and maintained brood number for accuracy of viral transmission.

Viral susceptibility on fire ant larvae

4th larvae were collected from healthy and SINV-1 infected colonies as confirmed by PCR detection. All samples were fixed overnight at 1% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.0). And, tissues were secondarily fixed with 1% in 0.05 M OsO4 solution for 1 hour. The fixed tissues were then dehydrated through serial acetone. Finally, 4th larvae embedded in Spurr resin (Spurr A. K., 1969). Tissues were identified through thick sections (1μm) with Toluidine blue O stain (TBO). And then, ultra-thin sections were survey on grids and stained with uranyl acetate. Thin sections were observed on Hitachi H-7650 (TC5 Bio-image Tools, NTU).
Result

Field surveying, and virus detection

SINV-1 infectious status, in Taiwan, has a widespread pattern, and so far infection rate from fields collected is 46% (Table 1). Among 165 nests collected, infection rates were variable across collection sites (0 – 100%). 4 of all 16 collection sites were re-collected two to three times; infection rates of re-collected site signified that distribution may increase viral transmissibility.

SINV-1 horizontal transmission

Results from horizontal transmission experiment revealed no infection detected after 14 days. However, the horizontal transmission was evident, most likely through trophallaxis from infected larvae to workers, in 3 of 5 replicates after 30 days.

Viral susceptibility on fire ant larvae

Healthy larvae revealed functional epithelial cell in alimentary canal (Fig. 1A and 1B). Midgut tissues showed cytopathic effects on infected fire ant larva (Fig. 1C). Cytoplasmic vacuoles from infected larva showed the processes of apoptosis in epithelial cell (Fig. 1D). Some of epithelial cells showed chromatin condensation and autolysis in alimentary canal. SINV-1 not only induced variation in epithelial cell, but also Malpighian tubes at some infected larvae. In ultra-structure of midgut cells, epithelial cell shows functional structure and full with microvilli in healthy larva (Fig 2). However, it reveals destruct cell ostensibly in infected larva. The epithelial cell from infected larva induced abnormal structure, cytoplasmic vacuoles, and impact reduction of microvilli (Fig. 3).

Discussion

SINV-1 has high transmissibility and persistent infection in fire ant colonies. In Taiwan, invasion sites almost located at highly disturbed environments that maybe increase frequency of inter-colony communication, and the chance of viral transmission. The territoriality of monogyny form may interfere in the transmissibility of pathogens (Oi et al., 2004). The pathways of viral transmission were feasible through trophallaxis directly in fire ant colonies. However, horizontal pathway seems not smooth at intra-colony level. Picornaviruses induce apoptosis death were reported between host-pathogen interactions (Buenz and Howe, 2006). The defensive response was often observed in host insect midgut after virus infected. (Vaidtanathan and Scott, 2006). However, viral proteins inhibits machine of apoptosis contrarily, and pathogens persistent infection in hosts (Koyama et al., 2001). Furthermore, highly infection rate and host defensive responses still have many problems to contend with.
Reference


Table 1  Survey of fire ant nests for the presence of SINV-1 in Taiwan

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Nests surveys</th>
<th>Infected nests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>06/22/2008</td>
<td>SW1</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>06/22/2008</td>
<td>SW2</td>
<td>11</td>
<td>64</td>
</tr>
<tr>
<td>07/24/2008</td>
<td>SW2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>08/05/2008</td>
<td>YM1</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>08/19/2008</td>
<td>CG</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>08/20/2008</td>
<td>YM2</td>
<td>18</td>
<td>39</td>
</tr>
<tr>
<td>09/19/2008</td>
<td>CG</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>09/19/2008</td>
<td>YM2</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td>11/13/2008</td>
<td>SW3</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>12/01/2008</td>
<td>BG</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>12/08/2008</td>
<td>DS</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>12/18/2008</td>
<td>YM2</td>
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<td>89</td>
</tr>
<tr>
<td>02/03/2009</td>
<td>CG</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>03/25/2009</td>
<td>SL</td>
<td>6</td>
<td>17</td>
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<tr>
<td>04/02/2009</td>
<td>SW1</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>08/13/2009</td>
<td>SW4</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>09/18/2009</td>
<td>BD</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>10/29/2009</td>
<td>YM3</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>11/30/2009</td>
<td>LT1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>12/11/2009</td>
<td>LT2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>01/17/2010</td>
<td>LT1</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>03/17/2010</td>
<td>GS</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 1  Semi-thin sections of epithelial cell. (A) Semi-thin sections of midgut of healthy larva. Scale bar = 100 μm. (B) Epithelial cell around the peritrophic membrane (PM) in healthy larva. Lipid droplets (black arrow). Scale bar = 100 μm. (C) Semi-thin sections of midgut of infected larva. Scale bar = 100 μm. (D) Epithelial cell around the peritrophic membrane (PM) in infected larva. Cytoplasmic vacuoles (white arrow) Scale bar = 10 μm.
Fig. 2. Examination of midgut epithelial cell from healthy larva. (A) Epithelial cell full with mitochondria and tight microvilli. Scale bar = 2 μm. (B) Active situation in healthy alimentary canal. Scale bar = 2 μm. M, mitochondria. Mv, microvilli.

Fig. 3. Examination of midgut epithelial cell from infected larva. (A) Epithelial cell reveals cytoplasmic vacuoles and reduced microvilli. Scale bar = 10 μm. (B) Cytoplasmic vacuoles around with nuclear. Scale bar = 0.5 μm. Cv, Cytoplasmic vacuoles. N, nuclear. Mv, microvilli.
Genetic variation of *Solenopsis invicta* virus 1 (SINV-1) to track the invasion history of its host, red imported fire ant

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Abstract

Recent bottlenecked populations are generally featured by low genetic diversity at neutral genetic markers because they may have undergone reduction in population size. With paucity of genetic variation, contemporary genetic structure of given populations might be too weak to be detected, and several demographic events, such as recent dispersal, might be hard to track. However, rapidly evolving pathogens (particularly for directly-transmitted ones) may provide a powerful approach to address population dynamic of the host in a more detailed fashion than the genetic marker from host’s genome, which itself has been affected by population bottleneck. The recent introductions by the red imported fire ant, *Solenopsis invicta*, into Taiwan, Hong Kong and China in last decades represent the case that neutral genetic markers may not be variable enough to reconstruct the invasion history as genetic bottleneck is evident for these introduced populations. Despite this potential constraint, widespread distribution of *Solenopsis invicta* virus-1 (SINV-1) across these populations may serve as additional genetic marker that compensates reduced host’s genetic variation. The present study therefore employs sequences of two viral genes, RNA-dependent RNA polymerase (RdRp) and capsid protein gene, to resolve the phylogenetic relationships among populations, and, combined with sequences generated from the USA and South America, the global invasion route of fire ant will also be revealed. Finer scale of phylogeny of SINV-1 in single population, particularly for Taoyuan, will be investigated to determine the most likely source of newly found colony outside the core area.

Keywords: biological invasion, capsid protein, colonization history, phylogeny, RdRp, SINV-1

Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* (Buren), native to South America, was introduced in Mobile, Alabama in 1918 (Callcott, 1996) and has subsequently colonized throughout the southeastern USA as well as several western states including New Mexico and California. In the first decade of 21st century, this pest ant species has been detected in New Zealand (Nattrass and Van derwoude, 2001), Australia (Henshaw et al., 2005), Taiwan (Huang et al., 2004) and China (Zhang et al. 2007). To determine the relationship among these recently introduced populations and genetic structure within population, several studies have used genetic markers that were generated from *S. invicta* itself (eg. mtDNA and microsatellite) to address these relevant issues. (Henshaw et al., 2005; Yang, 2008). However, genetic variation at these markers is largely reduced since all theses populations may have undergone recent bottleneck associated with reduction in population size and founder effects that might affect the resolution and sensitivity of these markers. It has been suggested that pathogens or parasites with shorter generation time and rapid evolution may provide information regarding host demographic history and population structure (Criccione, 2006). Further, more detailed evolutionary history of
host might be revealed by its pathogen that transmits by host-host contact (Biek, 2006). A previous study has shown that all these introduced populations were found to bear high prevalence of *Solenopsis invicta* virus 1 (SINV-1) (Yang *et al.*, 2010), and this virus was considered as one of members of a single strand RNA virus that has high mutation rate, thus offering a great chance to use it as a genetic tool to track the invasion history of the red imported fire ant. Also, the signal of within population dispersal might be assessed due to this advantage of SINV-1. This study is therefore to use sequences of two viral genes (capsid protein and RdRp genes) to investigate the phylogenetic relationship among these recently introduced populations.

**Materials and Methods**

**Source of samples**

All RIFA colonies in the study representing both social forms were collected from five recently invaded countries: USA (Columbia, AL, Austin, TX, Gainesville, FL, Birmingham, AL and Mobile, AL), Australia (Richland-Waco area), China (Guangdong province), Hong Kong and Taiwan (Taoyuan and Chiayi counties). In total, we sampled 60 colonies from USA, 92 colonies from Australia, 31 colonies from China, 27 colonies from Hong Kong and 171 colonies from Taiwan. Collected workers were stored in 95% alcohol and preserved in -20ºC.

**RNA extractions and determination of SINV-1 infection**

We used two-step reverse transcription PCR (RT-PCR) to confirm the infection status of SINV-1. For the first step, RNA was extracted with 15 to 20 individual ants by using TRIZOL reagent (Invitrogen, USA). The first strand cDNA of all RIFA colonies was synthesized and amplified by using SuperscriptIII reverse transcriptase (Invitrogen, USA) with oligo dT(18). Subsequence PCRs were carried out with virus specific primers including P517/P523 and P341/P343 (Valles, *et al.*, 2005; Valles, *et al.*, 2007) to detect SINV-1/1A infection with following PCR cycling program: 1 cycle at 95 ºC for 10 min, 35 cycles of 94 ºC for 30s, 56 ºC for 30 s, 68 ºC for 90 s, followed by a final elongation step of 68 ºC for 5 min. Colonies were considered infected when a visible band appeared with anticipated fragment size (600 bp for P517/P523, 1.3kb for P341/P343) after running the gel with positive controls (known virus-infected ants), negative controls (uninfected ants) and no template controls in each PCR batch.

**Sequence analysis**

Sequences were alignment by eye with the reference sequences from GenBank; Paup and Mega were used to perform Neighbor-Joining tree reconstruction.

**Result**

**Sinv-1 detection**

P517/P523 is the primary primer to detect SINV-1, which is complementary to P341/P343 given the fact that high mutation rate is the typical feature for the region amplified by
the latter one and therefore may allow false negative. More colonies with positive signal for SINV-1 by P517/P523 than by P341/P343 were found (Table 1). In all sample sites, there is no SINV-1 detection in Australia and, no SINV-1A infection was detected in any samples.

**Phylogenetic tree at global scale based on RdRp gene**

All samples can be preliminary divided in two groups: America (all USA samples) and Asia group (Taiwan, Hong Kong and China) (Fig. 1). With low bootstrap values supported, no clear relationship among populations in Asia group could be obtained.

**Phylogenetic tree at global scale based on capsid protein gene**

The result based on capsid protein gene is also similar with that based on RdRp gene. Two major clusters were found and correspond to two geographic groups, Asia and USA groups (Fig. 2).

**Phylogenetic tree of RdRp gene in Taiwan**

It is difficult to distinguish relationships between colonies in small geographic areas (Fig.3). Although these samples can be divided into two groups (FA11, FA33 and others), no clear geographic grouping could be found.

**Phylogenetic tree of capsid protein gene in Taiwan**

Unlike the clustering pattern from RdRp genes, three major clades were found in capsid protein gene phylogeny, and there is no concordance between trees generated by these two genes (Fig.4).

**Discussion**

Over all, when a new RIFA population is detected, we are able to preliminarily distinguish whether it was from other populations in Asia or America. In finer geographic scale, like in Taiwan, the power to determine which geographic region in core population could be referred as the potential source of newly found colonies might be weak given all these within-population movement are fairly recent events.

**Reference**


### Table 1 SINV-1 detection in 5 countries (mean % of surveyed infected)

<table>
<thead>
<tr>
<th>Country</th>
<th>Total samples</th>
<th>P517/P523 (RdRp)</th>
<th>P341/P343 (Capsid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>60</td>
<td>25 (42%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Australia</td>
<td>92</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>China</td>
<td>31</td>
<td>14 (14%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>27</td>
<td>9 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>Taiwan</td>
<td>171</td>
<td>93 (54%)</td>
<td>41 (24%)</td>
</tr>
</tbody>
</table>
Fig. 1  NJ tree showing that present samples with RdRp gene sequence can be divided in two groups, Asia and America. In Asia group, “FA” means samples from Taoyuan in Taiwan, “Si” means samples from Chayi in Taiwan, “M” means samples come from China and HK means samples from Hong Kong. Notes: circle means low bootstrap value.
Fig. 2  NJ tree showing that present samples with capsid gene sequence can be divided in two groups, Asia and America. In Asia group, “FA” means samples from Taoyuan in Taiwan, “Si” means samples from Chayi in Taiwan, “M” means samples from China.
Fig. 3 N-J tree showing that it is difficult to distinguish the relationships between these populations. “FA” means samples from Taoyuan County in Taiwan.
Fig. 4 N-J tree showing that it is also difficult to distinguish the relationships between these populations like using RdRp sequence. “FA” means samples come from Taoyuan in Taiwan.
Fire Ant Predation and the Effects of Fire Ant Management on White Grubs (Coleoptera: Scarabaeidae) in Turfgrass

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Abstract:

White grubs (Coleoptera: Scarabaeidae) are one of the most destructive pests of turfgrass. About 10 species or genera of white grubs are found in managed turfgrass in the U.S. and most are root feeders. Recent reports suggest that white grubs problems are increasing in the southeastern U.S. This may be due to the removal of imported fire ants *Solenopsis invicta* with insecticides on certain sites. Imported fire ants are important predators of many turfgrass pests that feed on the surface but little is know about predation in the soil. Selective removal of ants may result in outbreaks of white grubs and other turfgrass pests. This study evaluated whether the control and removal of *S. invicta* from turfgrass would result in an increase in white grubs, and the relative susceptibility of different life stages of turf-infesting scarabs to predation by *S. invicta*. On a golf course near Auburn, AL, 10 × 10 m plots of hybrid bermudagrass were treated with the granular insecticides bifenthrin or fipronil, or hydramethylnon applied as a bait. Fire ant abundance before and after treatment was determined using hotdog baits. Plots were infested with adult Japanese beetles and sampled in fall for grub abundance. Life stage susceptibility was determined by exposing grubs, Japanese beetle eggs, or adult Japanese beetles and Green June beetles to active fire ant mounds in separate trials. Life stages were exposed at 2 m from active mounds for 24 h and percent loss due to predation was determined. Removal of fire ants in the field did not result in an increase in white grubs relative to untreated control plots. Eggs were the most susceptible life stage and adults were the least susceptible to fire ant predation in mound challenges. In subsequent lab tests, grubs and adults were attacked and eaten. This suggests that these life stages are somehow escaping temporally or spatially under field conditions. Additional field and lab experiments are being conducted in 2010 to build up on these results.
Study of midgut bacteria in the red imported fire ant, *Solenopsis invicta* Büren

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Abstract

Ants are capable of building close associations with plants, insects, fungi and bacteria. Symbionts can provide essential nutrients to their insect host, however, the development of new molecular tools has allowed the discovery of new microorganisms that manipulate insect reproduction, development and even provide defense against parasitoids and pathogens. In this study we investigated the presence of bacteria inside the Red Imported Fire Ant (*Solenopsis invicta*) midgut using transmission electron microscopy and molecular tools. After isolation and culture of these bacteria, the molecular analysis revealed ten unique profiles which were identified to at least the genus level. PCR results showed that *Enterococcus* sp., *Kluyvera cryocrescens* and *Lactococcus garvieae* are the most abundant species, but they are not consistently found in all sites throughout the southeastern United States. Three bacterial strains were selected, genetically modified with the plasmid vector pZeoDsRed, and successfully reintroduced into fire ant colonies. Although most of the transformed bacteria were passed out in the meconium, DsRed fluorescence was still detected up to seven days after pupal emergence. We further demonstrated that nurses contributed to the spread of the transformed bacteria within the colony by feeding the meconium to naive larvae. Antibiotic treatment of fire ant colonies, under laboratory conditions, confirmed that the entire bacterial community played an important role in the queen’s ability to reproduce and affected the colony fitness. These results are the foundation for a red imported fire ant biological control program using endosymbiotic bacteria.
Ecology and Economics of Post-Hurricane Fire Ant Perturbations in Louisiana Sugarcane

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Sugarcane (Saccharum spp. hybrids) has historically been the number one field crop in Louisiana. It is a semi-perennial crop grown over a four to six-year rotation cycle, i.e. three to five crops are harvested from a single planting and then followed by a fallow year. In the Louisiana sugarcane agroecosystem, the sugarcane borer, Diatraea saccharalis (F.), is the most economically damaging herbivore. D. saccharalis management relies on properly timed applications of narrow-range minimum-risk insecticides and associated conservation of arthropod predators. In the D. saccharalis arthropod predator complex, red imported fire ants, Solenopsis invicta Buren, and spiders are first and second in importance, respectively. Predaceous beetles (ground, click, tiger, and rove beetles) and earwigs are also D. saccharalis predators although their impact has been quantified far less. In addition to predators, arthropods such as crickets also have been considered important in the agroecosystem because they represent additional diet for predators.

In September 2005, salt water from the Hurricane Rita storm surge flooded 12,000 to 16,000 ha of sugarcane production along the Gulf Coast. A 12-replication, four-treatment study comparing storm surge flooded and non-flooded plant and ratoon sugarcane fields was conducted in 2006 and 2007 to assess soil-associated arthropod abundance and diversity and D. saccharalis infestations.

A 3-fold reduction in red imported fire ants was associated with the storm surge whereas no substantial reduction in the abundance of other soil-associated arthropods was recorded eight or more months after the hurricane. Post-hurricane disruption of the predaceous red imported fire ants was associated with a 30% greater soil-associated arthropod diversity and an increase in D. saccharalis infestations. Higher D. saccharalis pressure associated with the storm surge resulted in higher sugarcane production costs (increased insecticide use) and lower revenues (increased D. saccharalis-related yield losses). From estimates obtained in our study, D. saccharalis-related economic losses for the 2006 growing season ranged from $1.9 million to $2.6 million for 7.4 to 9.8% of the Louisiana sugar production area impacted by the hurricane storm surge, respectively. For the 2006 growing season, the sugarcane gross farm value was $319.7 million. D. saccharalis-related economic losses caused by the storm surge therefore represented approximately 0.6-0.8% of the gross farm value, meaning that each percent of area impacted by the storm surge caused an approximate loss of 0.1% of the gross farm value.

The semi-perennial nature of sugarcane and use of narrow-range insecticides minimize arthropod disruptions, contributing to the relative ecological stability of the agroecosystem. However, the storm surge disrupted this agroecosystem. Fire ants were negatively affected to a greater extent than other soil-associated arthropods. Relieved from fire ant predation, the soil-associated arthropod complex and D. saccharalis populations recovered faster from the storm surge disruption. Despite its catastrophic impacts, Hurricane Rita offered the opportunity to
document how the areawide suppression of an aggressive arthropod predator such as the red imported fire ant could impact the sugarcane arthropod complex and enhance economic losses.

Efforts to Develop Pre-Harvest Treatments for Imported Fire Ant Quarantine Certification of Field-Grown Nursery Stock in Tennessee

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Introduction

Imported fire ants (Solenopsis spp.) (IFA) have numerous well-documented impacts to both humans and ecological systems. Since IFA are easily transported in soil-containing products, IFA can threaten the profitability of nurseries through trade restrictions, elimination of markets, and the imposition of costly, impractical, and unfeasible quarantine regulations. Based on market destination of several southern states, IFA quarantine treatments affect an estimated 80% of nursery plant sales, respectively. The 'green industry', which includes nurseries, has an estimated $150 billion dollar impact on the U.S. economy (Hall et al. 2005), and therefore, any trade disruptions imposed by IFA can have important economic implications.

Field-grown nurseries produce plants directly in field soil, and the plants are typically machine harvested from the ground and wrapped in burlap surrounded by metal baskets (i.e., balled and burlapped root balls [B&B]). Currently, only three IFA quarantine treatments are approved for field-grown nursery stock: 1) a pre-harvest broadcast bait (e.g., Amdro, Award, Distance, Extinguish, or Extinguish Plus) followed 3-5 days later with granular chlorpyrifos, 2) a post-harvest B&B dip in chlorpyrifos, or 3) a post-harvest twice daily for three consecutive days B&B drench in chlorpyrifos (USDA-APHIS 2007). The broadcast pre-harvest method is the most practical application method, but is cost prohibitive (~ $320 to $500 / treated acre). The post-harvest B&B dip or drench methods are labor intensive, impractical for treating large numbers of nursery plants, potentially damaging to the environment, hazardous to labor because large volumes of insecticide are used, and very expensive (~ $530 to $1,165 / acre of treated trees [equivalent to 1,037 trees / acre]). The approval of only one insecticide active ingredient threatens industry viability if chlorpyrifos becomes unavailable. Heavy reliance on chlorpyrifos for IFA quarantine treatments also poses an acute exposure hazard to agricultural workers because chlorpyrifos ranked third among pesticide active ingredients attributed to the largest number of acute-pesticide-related illnesses (Calvert et al. 2004). The U.S. Environmental Protection Agency cited chlorpyrifos treatment of B&B nursery plants as an area of concern for post-treatment handling risks to agricultural workers (USEPA 2000, 2001).

A short post-certification period for field-grown nursery stock is another problematic issue with the current IFA quarantine treatment protocols. The certification length for the post-harvest
B&B dip and drench protocols is 30 days and for the pre-harvest broadcast granular option is 84 days. The production system employed by field-grown nurseries, which requires plant harvesting during dormant periods (October to April) and accumulation of plants until peak market demand in the spring, does not function with short certification periods. The pre-harvest treatment requires an IFA bait treatment before the first granular chlorpyrifos treatment. IFA baits require warm weather for the IFA workers to effectively forage the bait, and therefore, bait treatments cannot be applied much later than October in most years. Since baits must be applied no later than October, and the granular chlorpyrifos in-field treatment only has an 84-day certification, this forces field-grown nurseries to make two granular applications per year to certify their plants for the entire harvesting season. Two granular chlorpyrifos applications are not only costly, but also excessively contaminate the environment with 12 lb active ingredient [AI] / acre. If growers do not ship their plants during these short certification periods, they must reapply the problematic dip, drench, or granular treatments.

The quarantine treatment protocol most needed by the field-grown nursery industry for IFA is a low cost pre-harvest fall treatment that has a certification period lasting through spring. The purpose of this study was to evaluate combinations of broadcast IFA baits with banded applications of pyrethroids to potentially provide longer IFA certifications at costs that are lower than current IFA quarantine protocols.

**Materials and Methods**

Treatments evaluated in Tennessee field-grown nursery settings were first developed by personnel at the USDA-APHIS Soil Inhabiting Pests Section, Gulfport, MS (SIPS) in turf settings. The most promising IFA treatments at SIPS have been broadcast IFA baits (i.e., hydramethylnon, fenoxycarb, pyriproxyfen, or [S]-methoprene) followed 3 to 5 days later with a 6-ft (1.8-m) wide band of a pyrethroid (e.g., bifenthrin, lambda-cyhalothrin, deltamethrin, or permethrin).

In Tennessee, field-grown nursery sites were located with sufficient IFA infestations to evaluate insecticide treatments. A sufficient infestation was considered a minimum of 5 colonies per plot, although most plots in this study had at least 10 IFA colonies. Although some black IFA (Solenopsis richteri Forel) were present at test sites, most IFA colonies were the Solenopsis invicta Buren × S. richteri hybrid. Tests were performed annually from 2005 through 2009. Treatments were initiated in the fall (September to November) and monitored through spring (April or May). Numerous treatments were evaluated, including 1) not baiting or pre-baiting with either broadcast Amdro, Award, Advion, or Extinguish Plus, 2) pyrethroid type (bifenthrin, lambda-cyhalothrin, or permethrin) applied at labeled rates, 3) pyrethroid frequency (1 or 2 applications), 4) pyrethroid timing (September, October, November, December, February, or March), 5) band widths ranging from 5 to 12 feet (1.5 to 3.7 m), 6) spray application volume (26 to 88 gallons / acre [243 to 823 liters / hectare]), and 7) nozzle type (flatfan versus floodjet). In 2009, protocols were modified to include either 1) a banded tank mix of lambda-cyhalothrin plus bifenthrin or 2) individual fire ant mound treatments (IMT) in combination with the broadcast bait and banded pyrethroid sprays. Mound size criteria used to determine if an IMT treatment would be applied included any mound with a diameter ≥ 20 cm in any axis or any mound with a height ≥ 10 cm. The mound size criterion was arbitrarily set as a size that would likely be readily
visible to a nursery worker from a moving tractor. All mounds meeting the size criterion, whether active or dormant, received the IMT treatment. The IMT treatments consisted of either a bifenthrin 23.4EC drench applied at a rate of 0.025 fl oz / gal (0.2 ml / liter) in 1 gallon (3.785 liter) solution or Advion Fire Ant Bait applied at a rate of 0.5 oz / 3 feet (14.2 g / 0.9 m) diameter area centered on the mound. The bifenthrin IMT was applied immediately after making five holes in the mound ~ 0.75 in (~1.9 cm) with a bamboo rod to a depth at least 4 in (10.2 cm). Plots were evaluated by performing pre-treatment counts of active IFA colonies, followed by bi-weekly to monthly monitoring to determine activity status of existing mounds and to look for new colonies entering the treatment zone. Mounds were checked for activity by inserting a 15-gauge (AWG system) wire into the mound in multiple locations and defined as active if at least 5 worker ants appeared.

Results and Discussion

Using broadcast baits and one to two pyrethroid band applications, management of IFA in Tennessee nursery sites has been variable and inconsistent. In contrast, USDA turf evaluations with broadcast baits and pyrethroid bands in Mississippi often achieved complete IFA control for 0.5 to 6 months. The major difference between states is the predominant clay soils in Tennessee and the sandy soils in Mississippi. The Tennessee site also differed in having primarily herbicide-killed vegetation between nursery plants within the nursery rows, as opposed to actively growing grass in Mississippi turf sites. Despite inconsistencies in control at Tennessee nursery sites, several findings were made from the project, including: a) two pyrethroid band sprays provided greater IFA control than one application (a single application was unacceptable), b) inclusion of broadcast IFA bait before the pyrethroid application provided greater overall IFA control than just using a pyrethroid alone, c) pyrethroid treatments that began later (October or later) were more effective than earlier sprays (September), which was attributed to increased winter rainfall, d) width of pyrethroid spray bands (5 ft [1.5 m], 6 ft [1.8 m], or 12 ft [3.7 m]) were not major factors in the level of IFA control achieved, e) total spray volumes (26 to 88 gal / acre [243 to 823 liters / hectare]) were not significant factors in IFA control, f) floodjet nozzles, which allow growers to spray both sides of the nursery row simultaneously, performed as well as traditional flatfan nozzles, g) pyrethroids like bifenthrin and lambda-cyhalothrin were very effective at preventing IFA re-infestation once existing IFA colonies were eliminated, as indicated by influxes of new IFA colonies into control plots, but not pyrethroid-treated plots, h) bifenthrin followed by lambda-cyhalothrin was the most effective treatment combination, suggesting alteration in pyrethroid types may be more effective than consecutive applications of the same pyrethroid, and i) IFA colonies that were ranked as large on a scale of 1 (small) to 10 (large) were more difficult to eliminate with baits and pyrethroid bands than smaller-sized colonies.

During 2009, modifications to treatments previously evaluated included either banded applications of tank mixed bifenthrin and lambda-cyhalothrin or IMT treatments (bifenthrin drench or Advion) applied to mounds defined as 'large in size' by criteria previously described. Extinguish Plus broadcast bait was used in advance of all treatments evaluated in 2009. All treatments also received pyrethroid bands either individually or as tank mixes with some treatments also receiving IMT treatments. All treatments that received the IMT bifenthrin drench achieved rapid IFA control (98%) within 1 week following treatment. Treatments receiving the
tank mix of bifenthrin and lambda-cyhalothrin only achieved rapid control when a bifenthrin IMT was also included. All treatments, with the exception of the Advion IMT, had 100% IFA control by 13 weeks after treatment. The Advion IMT treatment had 100% IFA control at 20 weeks after treatment. Extreme cold weather between 13 and 20 weeks after treatment did not allow monitoring of the test, so it is possible the Advion treatment also achieved 100% control earlier than 20 weeks. The cold weather during the 2009 trial also affected the non-treated control plots, which had 46 total active colonies at pre-treatment time (early October), 58 active colonies at 8 weeks (mid-December), 34 active mounds at 13 weeks (mid-January) before the extreme cold weather began, and 20 and 9 active colonies at 20 and 22 weeks after treatment (early and late March), respectively. The 2009 trial is still being monitored during spring 2010 to determine how long test plots will remain IFA free. To date, the results indicate IMT treatments provided a substantial advantage in the elimination of large colonies not readily removed by pyrethroid bands alone. Now that all treated nursery plots are IFA free, it is hoped the pyrethroid bands will continue to demonstrate the ability to prevent re-infestation of test plots under current evaluation. The cost of treatments in this test are estimated at $59 to $186 / treated acre, which is about one-half the estimated cost of the lowest chlorpyrifos treatment currently available.

References Cited


Coachella Valley Golf Course Red Imported Fire Ant Treatment Program Evaluation

Bastiaan “Bart” M. Drees, Professor and Extension Entomologist, Texas AgriLife Extension Service, Texas A&M System, College Station, Texas, and Roberta “Bobbye” Dieckmann, Biologist, Coachella Valley Mosquito and Vector Control District, Indio, California

Until 2009, the treatment program for golf courses and other areas infested with the red imported fire ant (RIFA), *Solenopsis invicta* (Hymenoptera: Formicidae) by the Coachella Valley Mosquito and Vector Control District (CVMVCD) was outlined in the Red Imported Fire Ant 2007 Standard Operating Procedures document. The standard program relied on an initial broadcast application of an insect growth regulator (IGR) bait product (e.g., *Distance*® containing pyriproxyfen, or *Extinguish*® containing methoprene) followed in approximately 10 days with a fast-acting bait (e.g., *Advion*® containing indoxacarb). After approximately 3 months, the site was to be re-assessed using a food lure bait (i.e., Frito® corn chips placed in a plastic vial under a shade structure) and spot treatments made only to those locations where ants were attracted to the food lure.

This treatment regime was based on the rationale for achieving eradication by the California Department of Food and Agriculture (CDFA) as discussed in Drees et al. 2000. The regime focused on treating all active *S. invicta* colonies with an IGR so to prevent further spread through mating flights. However, currently only a fraction of the 183 or so golf courses in the Coachella Valley is under this treatment program, so successful mating flights currently persist in the area. Furthermore, the IGR treatment is slow-acting. Alternative treatment sequences include 1) applying a faster-acting bait such as *Advion*® containing indoxacarb first and waiting for ant activity to resume in a month or more before applying the IGR such as *Distance* containing pyriproxyfen to maintain suppression; or 2) applying the faster-acting bait product (indoxacarb) first or using a mixture or tandem treatment of both ingredients (e.g., *Extinguish*® Plus containing hydramethylnon plus methoprene, or *Advion*® blended or applied with *Distance*® at the same time at half rates of each product) for an initial treatment.

This study compared the *Distance*®-Advion® and Advion®-Distance® treatment sequences to treatment sequences using Advion® or Extinguish® Plus only. The objective was to assess the impact of the current treatment program as compared to alternative treatment sequence programs in a replicated and controlled design.

**Materials and Methods**

This effort required 16 golf courses being contracted for treatment in 2008 and 2009. Four replications of treatment programs were established. Treatment sequences could included: 1) Standard SOP treatment (*Distance*® followed 1 month later by Advion®); 2) Advion followed in 1-2 months with Distance; 3) “hopper blend” using Extinguish®; and, 4) Advion, only (*Appendix 1*). All broadcast treatments were applied using Herd GT77 model seeder.

In order to have a basis for comparison and add untreated “control” groups, each property coming under contract involved: 1) a pre-treatment survey; and, 2) an area set aside in each
property to serve as a “golf course standard” that would either be an “untreated or control area” or an area being treated by golf course personnel in a manner existing prior to the contract. Thus, a section designated as a “control” was established and maintained untreated by CVMVCD during the course of this assessment for a year for initial treatment results and thereafter as an assessment of “maintenance” or no treatment.

The first four golf course properties established during the course of this study received one of each of these treatment regimes assigned randomly and became the first replicate. The second set of four properties would be randomly assigned one of each of the four treatments became the second replicate and so forth. Thus, initiation of treatment regimes were replicated and initiated seasonally during the course of the year, resulting in the ability to analyze for effects of when applications were initiated during different times of the year (e.g., avoiding summer and winter months with bait applications).

Ant foraging and mound number monitoring methods: Because *S. invicta* colonies in Coachella Valley golf courses occur largely next to the base of tree trunks, sand pits, and sources of moisture such a sprinkler heads, the method used for assessing mound numbers consisted of using a ¾ inch diameter, 3 ft long dowel rod to disturb soil in these areas (a process called “rodding”) and the number of times active imported fire ants were observed versus the total number of potential nesting sites was documented. For instance, if 40 tree trunk bases are disturbed and 30 are found with active ant mounds, the level of infestation could be calculated to be 75%. In subsequent visits, if only 10 active mounds were detected when disturbing 40 tree bases (ideally the same trees), the level of infestation could be said to have been reduced to 25%. Similar sets of 40, 60 or more (preferred) for assessment were made and results documented. For each 18 hole golf course, 180 “rodding” hits were taken to monitor for percent active colonies (10 per tee, 10 per fairway and 10 per green = 30 per hole times 6 holes). For untreated control areas from 10 to 30 “rodding” hits were taken. An effort was made to be consistent between areas and over time during all pre-treatment assessment dates. This procedure was done prior to any treatments both in the area to be treated as well as the area being set aside as the “control” (golf course “standard”) treatment area. Thus, a “pre-treatment control” as well as a “side-by-side control” area was set up for each golf course property in this study.

Post-treatment program initiation evaluations were conducted in the same manner as the pre-treatment assessment described above, and for each post-treatment program assessment date. The time sequence for post-treatment assessment(s) was consistent among all golf course properties at 3 weeks, 2 months, 4 months and 8 months following program initiation.

Treatment means (number of positive “hits” or active red imported fire ant colonies detected by tapping suspected nests with a dowel rod on 16 golf course grounds, prior to and periodically (3 weeks, 2 and 4 months) following program initiation for each treatment regime from the replicate golf course properties, were converted to arcsine values for analysis of variance (ANOVA) at $P < 0.05$, with means separated using Duncan’s Multiple Range Test, at $P < 0.05$ (SPSS for Windows, Version 17.0).

With untreated control plots established in each golf course participating in this study, corrected percent control values could be calculated using Henderson-Tilton’s formula:
Corrected % = \frac{n \text{ in } Co \text{ before treatment} \times n \text{ in } T \text{ after treatment}}{n \text{ in } Co \text{ after treatment} \times n \text{ in } T \text{ before treatment}} \times 100

Where: \( n \) = percent active ant mounds, \( T \) = treatment plot data, and \( Co \) = untreated control plot data. Negative percent control values were replaced with zero when graphing mean values for each treatment regime.

Results and Discussion

The golf courses used in this study, and the pre- and post-treatment program initiation results from ant mound monitoring efforts were used to assess the effect of treatment program or sequence and the effects of initiating programs during different times of the year (Table 1). In this first analysis, “untreated control” area data were not presented on analyzed. Statistical analysis of the arcsine values of mean percent active mounds for all four replicates of treatment programs indicated no significant differences in pre-treatment means, and that application of Advion® by itself resulted in the fastest numerical reduction of active ant mounds in treated areas although reduction was only significantly different from application of Distance® followed by Advion® (Table 2, Fig. 1). This effect was expected because at the time of the 3 week post-treatment assessment, only the insect growth regulator (IGR), Distance®, had been applied and effects of an IGR treatment is not evident for 1 to 2 months following application.

No significant differences occurred at 2 months following program initiation (Note: for the Extinguish® Plus data set, there was missing data from PGA West Palmer, so the men of the other three courses was used there for analysis). At 4 months after treatment program initiation, the Extinguish® Plus treatment program had produced significantly fewer active ant mounds than the Advion®-Distance® treatment program sequence, nut only numerically lower active ant mound numbers than the other two treatment programs.

Analysis of initiating these four programs during the winter months versus spring revealed that the pre-treatment percent active ant mound mean was greater in spring, but that the spring-initiated programs provided numerically better suppression at 2 months following program initiation (Fig. 2). However, active mound numbers began increasing thereafter in spring-initiated programs while winter-initiated programs seemed to be stable.

Using untreated control plot data from each participating golf course, the corrected percent control values were calculated for each (Appendix 2) and means graphed (Fig. 3). Results varied greatly among the golf courses even for a single treatment regime and may have resulted from treatment coverage, irrigation following application, or other issues rather than from the active ingredients used. Results differ from those using no untreated control plot data because percent active colonies in these areas on some golf courses declined over the course of the study and fewer samples (“rod hits”) were taken in some locations. However, of note is the initially low percent control for the Distance-Esteem treatment at 3 weeks after treatment as expected for a juvenoid IGR product’s performance. By 2 months following treatment initiation, the Advion® (indoxacarb) had been applied and percent control exceeded those of other
treatment regimes until the 4 month evaluations. Extinguish® Plus (hydramethylnon plus methoprene) performed consistently, providing roughly 65% control throughout the monitoring period. The performance trends are important in these results, more so than the actual percent control, which was intentionally sampled using the sites of ant mounds. Results of treatment is open areas (where golfer’s are located) may likely have been better that at likely nesting sites where sampling occurred.

This study will continue to attain 8 month post-treatment data, and final analysis will incorporated data obtained from untreated control plots established and monitored within each participating golf course.

On March 2, 2009, only 6 months after the initiation of this study, the RIFA program on golf courses being implemented by CVMVCD officially changed the SOP to implement the Advion® followed by Distance® treatment program (Memorandum to RIFA Operations Crew by Jim Saulnier: “Effective immediately, the initial treatment would be made using Advion®, followed 1 month later with the insect growth regulator (IGR), Distance®”). This decision was based, in part, on discussions between Drees and field staff and remains the subject of the golf course program monitoring program.

Acknowledgements

The authors wish to thank the golf course cooperators in this study, Jim Saulnier, Phil Boeing, William Wolf, Jeremy Wittie, and the entire Coachella Valley Mosquito and Vector Control District (CVMVCD) team of technicians in the red imported fire ant program. We would also like to thank the CVMVCD Board of Trustees for funding this effort.

Citations

Table 1. Golf course participation in program assessment, and percent positive “hits” or active red imported fire ant colonies detected by tapping suspected nests with a dowel rod on 16 golf course grounds, prior to and periodically (3 weeks, 2 and 4 months) following program initiation, Coachella Valley, Riverside County, California, Oct. 2008 – Dec. 2009.

<table>
<thead>
<tr>
<th>NO.</th>
<th>GOLF COURSE</th>
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<th>2 months</th>
<th>4 months</th>
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<tbody>
<tr>
<td></td>
<td>ADVION®-DISTANCE®</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>Indian Ridge Grove</td>
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<td>27.70</td>
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<td>2</td>
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<tr>
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<td>11.63</td>
<td>41.06</td>
</tr>
<tr>
<td></td>
<td>MEAN</td>
<td>48.20</td>
<td>22.23</td>
<td>20.24</td>
<td>32.64</td>
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</tbody>
</table>

|     | ADVION                       |                       |         |          |          |
| 1   | Seven Lakes                  | 33.95                 | 6.83    | 2.20     | 2.20     |
| 2   | Indian Canyons North         | 70.55                 | 9.97    | 11.08    | 45.53    |
| 3   | Rancho La Quinta Jones       | 66.08                 | 7.75    | 14.98    | 7.18     |
| 4   | PGA West Nick Tournament     | 62.20                 | 13.28   | 9.42     | 12.2     |
|     | MEAN                         | 58.20                 | 9.458   | 9.42     | 16.78    |

|     | DISTANCE-ADVION              |                       |         |          |          |
| 1   | Outdoor Resorts              | 45.14                 | 28.11   | 9.97     | 9.22     |
| 2   | Tahquitz Resort              | 56.63                 | 47.2    | 11.07    | 21.63    |
| 3   | Tahquitz Legends             | 42.72                 | 30.53   | 4.97     | 14.40    |
| 4   | PGA West Weiskopf            | 34.97                 | 18.85   | 8.85     | 21.08    |
|     | MEAN                         | 44.87                 | 31.17   | 8.72     | 16.58    |

|     | EXTINGUISH® PLUS             |                       |         |          |          |
| 1   | PGA West Palmer              | 37.77                 | --      | 29.93    | 22.18    |
| 2   | Rancho La Quinta Pate        | 33.30                 | 7.75    | 3.87     | 5.53     |
| 3   | PGA West Nick Private        | 30.53                 | 12.75   | 6.65     | 9.43     |
| 4   | PGA West Stadium             | 45.33                 | 21.07   | 11.08    | 15.52    |
|     | MEAN                         | 36.73                 | 13.86   | 12.88    | 13.17    |
Table 2. Mean percent positive “hits” or active red imported fire ant colonies detected by tapping suspected nests with a dowel rod on 16 golf course grounds, prior to and periodically (3 weeks, 2 and 4 months) following program initiation, Coachella Valley, Riverside County, California, Oct. 2008 – Dec. 2009.

<table>
<thead>
<tr>
<th></th>
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<th>3 weeks(^1)</th>
<th>2 months</th>
<th>4 months(^2)</th>
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<td>ADVION®-DISTANCE®</td>
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<td>28.66a</td>
</tr>
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<td>9.46a</td>
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<td>13.86ab</td>
<td>12.88</td>
<td>13.17b</td>
</tr>
</tbody>
</table>

\(^1\) Means (percent values shown) in columns followed by the same letter are not significantly different using Analysis of Variance (ANOVA) performed on arcsine converted percentage values, with means separated using Duncan’s Multiple Range Test at \(P < 0.05\) level (\(F = 4.549\); d. f. = 3; Mean Square (Error) = 0.009).

\(^2\) Means (percent values shown) in columns followed by the same letter are not significantly different using Analysis of Variance (ANOVA) performed on arcsine converted percentage values, with means separated using Duncan’s Multiple Range Test at \(P < 0.05\) level (\(F = 2.326\); d. f. = 3; Mean Square (Error) = 0.014).

Figure 1. Treatment program impact: Mean number (percent) of positive “hits” or active red imported fire ant colonies (detected by tapping suspected nests with a dowel rod) of four different treatment programs or product application sequences (employing broadcast application of different sequences of granular bait-formulated products) on 16 golf course grounds, with sets or replicates initiated seasonally and randomly assigned selected treatment programs, Coachella Valley, Riverside County, California, Oct. 2008 – Dec. 2009.
Figure 2. Seasonal program initiation impact: Mean number (arcsine of percentage) of positive “hits” or active red imported fire ant colonies (detected by tapping suspected nests with a dowel rod) of all treatment programs or product application sequences on 16 golf course grounds, comparing a set initiated in the winter (Oct. 3, 2008, Jan. 30, 2008, Nov. 4, 2008, and Oct. 8, 2008) versus spring (May 12, May 19, May 19, and May 19, 2009), Coachella Valley, Riverside County, California, Oct. 2008 – Dec. 2009.
Broadcast and Skip Swath Bait Treatment Effects on Fire Ant Mound Height and Density  
Victoria County, Texas - 2008-2009  
Charles L. Barr, Barr Research and Consulting  
Sam Womble, CEA, Victoria County

Abstract

Large fire ant (*Solenopsis invicta* Buren) mounds can cause considerable damage and monetary loss in hay pastures. There are three potentially cost-effective means of controlling them: broadcast baits, mechanical dragging, or a combination of the two. Results of this trial indicate that all three methods have their benefits. The combination was the most effective method with mound heights kept to an average of 2.25 inches tall compared to 5.6 inches in untreated plots a year after treatment. Displaced soil volume was many times greater in undragged plots than in bait-treated, dragged or combination plots. With a typical cutting height of three to four inches, hay harvesting would be largely unaffected in treated, dragged plots, but difficult in untreated plots. The choice of which method to use depends largely on whether ant presence or mound presence, or both, is the real problem.

Introduction

Red imported fire ants (*Solenopsis invicta* Buren) cause numerous problems in cattle and hay production systems. Problems such as electrical failures and stinging incidents are directly related to the presence of the ants themselves. However, particularly in hay pastures, the ants’ mounds cause most of the problems. Large mounds jam cutters and balers, dull blades and can result in substantially lower equipment speed and increased wear and tear due to the roughness of the ground. Mounds are a particular problem in heavy clay soils where they are overgrown by grass and become an almost permanent part of the landscape.

There are two potentially cost-effective methods for resolving these problems - baits and dragging. Broadcast baits can reduce fire ant populations by over 90% in a single application at a cost of roughly $12 per acre (product cost only). Baits will solve most of the ant-related problems, but do little to solve the problems caused by mound structures. Mechanical dragging, on the other hand, effectively eliminates the mound-caused problems, but does little to kill the ants, which will immediately begin to rebuild their mounds. This trial was designed to test the effectiveness of both methods singly and in combination using two different broadcast baits - Esteem and Extinguish Plus.

Materials and Methods

The trial was located on the property of Dr. Ray Smith in Victoria County, Texas in the Texas Coastal Plain. The site is in the flood plain of the Colorado River and has heavy, black clay soil. Standing water is common after heavy rains and, in response, the ants have built very large mounds. The site was grazed, and had a good cover of well-managed native grasses.

One purpose of the trial was to replicate real-life management practices using the
cooperator’s equipment under his operation. Consequently, test plots were large (an average of about 5 acres) and could not be replicated. Treatments included: Esteem (0.5% pyriproxyfen) and Extinguish Plus (0.36% hydramethylnon, 0.25% s-methoprene) and an untreated control. Both baits were applied at a rate of 1.5 lbs./acre using a Herd GT-77 seeder. Each treatment was applied to two plots. One plot was full coverage, the other was a skip swath application on approximately 50 foot centers, resulting in 0.75 lbs/acre total application weight.

Finally, half of each of the five plots was dragged. Because of the size and permanence of the mounds, dragging was not an easy task. After several attempts, the owner finally resorted to using the front-loader bucket of his tractor to knock down the mounds followed by dragging with a pin harrow to get them flush with ground level.

Pre-counts were conducted on May 14, 2008. High winds that would have made the skip swath applications very inaccurate prevented bait application until May 31. Evaluations were then conducted on July 3, 2008, January 8, 2009 and June 5, 2009.

Because of the need to mechanically drag half of each plot and ongoing grazing pressure, it would have been very difficult to accurately establish permanent marks. It was therefore decided to sample using random transects. Each transect was 150 ft. long and 30 feet wide, giving a sample area of 0.103 acres. Transects ran perpendicular to the bait application swaths so as to encounter as many “skips” as possible in those two plots. For each transect, the approximate diameter and height of each mound was measured with a ruler or tape measure and recorded, along with whether the mound was occupied by ants.

Results and Discussion

The mounds in this trial were truly enormous, but not atypical of mounds found in heavy clay soils. The average mound at pre-count was over 25 inches in diameter and over 10 inches tall. Dozens of mounds were 36 inches in diameter and some were over 48 inches. Many were over 14 inches tall with some as tall as 18 inches. The mounds made the pastures unsightly, to say the least, and they were actually dangerous to drive any vehicle over at more than five or six miles per hour. Because of the large size and low density, it was assumed that the majority of colonies were monogyne (single queen).

The region had been plagued by low rainfall for some months at the time of applications and conditions only worsened. By spring 2009, the area was rated as in “exceptional” drought, the worst rating. The drought undoubtedly affected the ants. Many of the mounds rated as “live” did, indeed, have a colony living in the mound structure, but the ants actually occupied only a small fraction of the mound. For instance, a mound 36 inches in diameter might have a pocket of worked soil only 6 inches in diameter during the January evaluation. At the July evaluation, there was no freshly worked soil whatsoever, so alive-dead ratings were highly suspect.

The drought only worsened into 2009, reaching historic proportions. With luck and a close eye on the weather radar, the June 5 evaluation was conducted two days after an isolated thunderstorm moved over the test site. Mound building was good and the vegetation grazed very short making for excellent visibility. Mounds of only a few inches in height and diameter were
easily spotted.

Because the treatments were not truly replicated, it was inappropriate to analyze them using statistical methods such as analysis of variance. Fortunately, many of the differences were well illustrated by simple summary statistics. One challenge of the analysis was finding a way to express the number, condition (alive or dead) and size (height and diameter) of the mounds with a simple expression that could be easily compared between treatments. Mound counts or densities did not express mound size. Average heights did not take density into account; i.e. 10 mounds could have the same average height as 100 mounds.

It was finally decided that it was best to separate bait effectiveness and dragging. Bait effectiveness was expressed simply by the total number of live mounds in both dragged and undragged sub-plots of each treatment, as shown in Table 1. Because the plots were not replicated, there was no way to equalize any pre-count factor so substantial differences existed between plots at test initiation.

Comparison of dragging effectiveness was more complicated. The mound structure itself causes the most damage so it was not necessary to differentiate between occupied (live) or abandoned mounds for this comparison. Mound height is the most important factor in equipment damage, but diameter also plays a role. For example, a mound 12 inches high and 36 inches in diameter is a much more potentially damaging structure than a mound of the same 12-inch height but only 18 inches in diameter.

Therefore, it was decided that mound volume might be the most accurate and useful comparison. Volume was calculated using the formula for the volume of a partially filled sphere:

\[ \frac{2}{3} \pi h^2 (r-h/3). \]

The major assumption was that mounds are spherical which, of course, they are not, but it was the best fit of any regular geometric shape. As mentioned, averages did not take mound number into account, so the volumes of all mounds were simply summed. Table 2 shows the results of dragging the different treatments.

It was expected that Extinguish Plus, because of its faster-acting hydramethylnon component, would eliminate colonies considerably faster than the IGR-only Esteem. However, as shown in Table 1, there were few differences in effectiveness between the two baits at both Week 5 (July) and Week 33 (January). The drought was the likely reason because it both caused the Esteem-treated ants to die faster than usual and resulted in so little freshly worked soil that existing mounds were very difficult to find. By January, a total of only seven active mounds were found in all four bait-treated plots.
Table 1. Total number of mounds per three 150 x 30 ft. transects. (Total area = 0.31 acres)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-count</th>
<th>Week 5</th>
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<th>Week 52</th>
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<tr>
<td></td>
<td>Live</td>
<td>Tot</td>
<td>Live</td>
<td>Tot</td>
</tr>
<tr>
<td>Untreated - undragged</td>
<td>15</td>
<td>29</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>- dragged</td>
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<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Ext. Plus, full coverage - undragged</td>
<td>19</td>
<td>34</td>
<td>4</td>
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<tr>
<td>- dragged</td>
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<tr>
<td>Esteem, full coverage - undragged</td>
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<td>- dragged</td>
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<td>29</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>- dragged</td>
<td>17</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Esteem Skip Swath       - undragged</td>
<td>6</td>
<td>23</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>- dragged</td>
<td>17</td>
<td></td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

At Week 5, there were roughly double the number of active mounds in the skip-swath plots than in their full-coverage counterparts. It is unknown whether these differences were statistically significant or the result of not being able to fully replicate treatments. After disappearing in the January evaluation, the differences reappeared at one year, but only in the Extinguish Plus plots. The number of active mounds were similar between the Esteem full coverage and skip-swath plots. Extinguish Plus was the only treatment to show substantially fewer active mounds at one year post-treatment.

The results of dragging (Table 2) showed the expected dramatic differences. In the dragged halves of the plots, there were almost no mounds to be seen. There were large areas of bare soil where the mounds had once been, but nothing tall enough to damage equipment. Most former mounds, in fact, were depressed an inch or two because of their collapsed underground galleries. By January, despite the drought, vegetation had begun to overgrow many of these bare patches.

Mounds in the dragged half of the untreated plot had begun to rebuild by January while there was no rebuilding in any of the treated plots. By one year post-treatment, the differences were stark. None of the treated, dragged plots had more than 0.5 ft³ of soil displaced while the ants had moved 5.47 ft³ of soil in the untreated, dragged plot. This was an obvious indicator of the effectiveness of bait treatments on mound formation. There was also considerably more displaced soil in the skip-swath plots versus full coverage, though these differences were probably due to the larger pre-count volume.

It is interesting to note the actual volume of soil displaced by fire ant activity. The average for all plots at pre-count was 2.35 yd³ with a range of 1.29 yd³ to 4.18 yd³. Two or three cubic yards of soil spread across one-third of an acre is not very much, a layer a millimeter or two thick. However, that displaced soil comes from underground tunnels dug by the ants. These tunnels provide paths for increased aeration and water infiltration which may be quite beneficial, especially in heavy clay soils. The excavated soil may also have better structure and move nutrients to the soil surface. This observation only adds to the importance of analyzing the costs...
and benefits of fire ant treatments.

**Table 2.** Total mound volume per plot, in cubic feet (ft.³) using formula: \( (h^2 (r-h/3))/1728 \). Three 150 x 30 ft. transects per plot. (Total area = 0.31 acres)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-count</th>
<th>Week 5</th>
<th>Week 33</th>
<th>Week 52</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drag</td>
<td>Undrg</td>
<td>Drag</td>
<td>Undrg</td>
</tr>
<tr>
<td>Untreated</td>
<td>62.0</td>
<td>98.6</td>
<td>0.4</td>
<td>45.6</td>
</tr>
<tr>
<td>Exting. Plus, full coverage</td>
<td>40.7</td>
<td>42.4</td>
<td>0.1</td>
<td>63.8</td>
</tr>
<tr>
<td>Esteem, full coverage</td>
<td>38.4</td>
<td>34.9</td>
<td>0.1</td>
<td>41.4</td>
</tr>
<tr>
<td>Exting. Plus Skip Swath</td>
<td>61.4</td>
<td>60.4</td>
<td>0.1</td>
<td>60.6</td>
</tr>
<tr>
<td>Esteem Skip Swath</td>
<td>83.1</td>
<td>112.9</td>
<td>0.1</td>
<td>65.4</td>
</tr>
</tbody>
</table>

**Tables 3 and Table 4** list the average mound diameter and height in the plots, dragged versus undragged. The alive and dead mound data were summed. At week 33, there were simply no mounds, alive or dead, in the treated plots. Not surprisingly, the dragged half-plots had substantially lower mound heights than the undragged half. Even after dragging, however, mounds rebuilt to some extent in the untreated plot. This observation alone might justify treatment in areas where mound height (or visibility) is important, such as hay fields and ornamental turf.

At one year post-treatment, there were still substantial differences between the dragged and undragged half-plots, particularly in average mound diameter. It was very evident that mounds in the treated, dragged plots were the result of re-invasion. The same was only partially true in undragged half-plots as some colonies had re-inhabited existing mound structures. Mound heights declined in undragged plots due to weathering and trampling.

**Table 3.** Mean mound diameter (in.). Three 150 x 30 ft. transects per plot. (Total area = 0.31 ac.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-count</th>
<th>Week 5</th>
<th>Week 33</th>
<th>Week 52</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drag</td>
<td>Undrg</td>
<td>Drag</td>
<td>Undrg</td>
</tr>
<tr>
<td>Untreated</td>
<td>24.7</td>
<td>28.5</td>
<td>12.0</td>
<td>24.6</td>
</tr>
<tr>
<td>Exting. Plus, full coverage</td>
<td>19.6</td>
<td>24.7</td>
<td>-</td>
<td>28.1</td>
</tr>
<tr>
<td>Esteem, full coverage</td>
<td>26.9</td>
<td>29.1</td>
<td>-</td>
<td>28.2</td>
</tr>
<tr>
<td>Exting. Plus Skip Swath</td>
<td>26.1</td>
<td>25.6</td>
<td>6.0</td>
<td>29.6</td>
</tr>
<tr>
<td>Esteem Skip Swath</td>
<td>23.3</td>
<td>32.3</td>
<td>6.5</td>
<td>30.4</td>
</tr>
</tbody>
</table>
The overall goal of this trial was to evaluate the effectiveness of both bait treatment, dragging and the combination of the two on mound characteristics. Bait treatments, regardless of type or application method, reduced mound numbers very effectively. A bait treatment alone might be sufficient on land where the presence of ants is the critical factor, such as turf harvesting and recreational use.

Dragging unquestionably took care of mound height issues and might prove valuable for land uses such as hay harvesting, vehicular movement and ornamental turf. (Without human traffic, as long as turf looks good, it doesn’t matter whether ants are present or not.)

The real question is whether the combination of the two is useful. Dragging has little effect on bait unless it disrupts collection by dragging too soon before or after bait application. However, the data show that a bait application can substantially reduce mound-related problems in the long term. At one year, despite the recovery of colony numbers to pre-count levels (Table 1), displaced soil volume was still 50 to 100 times less in treated, dragged plots versus undragged. In untreated plots, the difference was only a factor of five (Table 2). Mound diameter in treated plots was half to one-fourth the diameter in undragged plots (Table 3) and heights were less than half (Table 4). In untreated plots, diameter and height were only about 30% less.

Critically for hay production, the average mound height at one year post-treatment in treated, dragged plots was 2.25 inches while in untreated plots it was 5.6 inches. Most hay is cut at a height of three or four inches. Therefore, mounds in dragged, treated plots were still low enough to not interfere with most haying activities. Mounds in undragged plots were still high enough to cause problems.

In summary, fire ant treatment option depends on land use. If the ants themselves are the problem, then semi-annual application of a broadcast bait is the better choice. If the mounds are the problem, dragging is the better choice and its effectiveness and duration is greatly enhanced by bait application.

Finally, we would like to extend our deepest thanks to Dr. Smith for allowing us to use his property and for the considerable time and effort he spent in applying the baits and, particularly, flattening the hundreds of huge mounds in the dragged halves of the plots.
Gaps in Knowledge in Fire Ant Research and Extension

Linda M. Hooper-Bùi, Kathy Flanders, Jian Chen, Xing Ping Hu, and Ashley Miller

The red imported fire ant, *Solenopsis invicta*, (RIFA) has plagued the Southeastern United States for many years. These ants have spread in the United States and have invaded and continue to invade other parts of the world. Scientists, entomologists, pest management professionals, biologists, and research cooperators have spent countless hours and energy working to both solve the problem of the RIFA and gain better understanding to its life history and effects on the environment and other species. Public education and services such as the eXtension.org website have been created to assist the public in dealing with problems related to RIFA, yet gaps in knowledge still remain. A review of eXtension.org website FAQs and an exhaustive review of peer-reviewed literature gave us insight into areas of RIFA research that contain these knowledge gaps and where future research can be focused. The FAQs represent a public mandate for this information to be obtained. Several areas of research need are: (1) whether fire ants cause fish kills, (2) repellents of fire ants new and old, (3) depth of fire ant mounds in all areas of invasion, (4) whether to disturb a mound when applying bait or contact insecticide, and (5) colony identification, tracking, and movement. Additionally, the public requests on eXtension.org mandate production of an easy-to-use guide for quickly identifying fire ants and their mounds.

**Fish kills**

One of the widely-known, yet misunderstood, gaps in knowledge is the effect of RIFA on fish. Anecdotal evidence and preliminary research is available for fish kills, but a definitive answer has not been found. The data in the literature to date is anecdotal at best. Research to document this phenomenon is difficult to conduct.

Other than the direct effects of ingesting fire ants, the most obvious concern in regards to fish kill is the effect of pesticides on aquatic life. Butler (1971) expressed concerns over potential for otherwise insoluble pesticides to be absorbed by other organic materials and carry into estuaries via runoff from rainfall. Concern has also been expressed in pesticides’ ability to leach into soil and contaminate groundwater (Butler 1971, Lee and Maruya 2006.) Blu Buhs (2004: 94-106 and references therein) documents fish and other vertebrate deaths but mostly attributed them to chemicals used in failed eradication attempts. This could allow contamination of not only ponds and waterways but also contamination of plant life growing in these areas. Kabishima et al (2004) investigated bifenthrin runoff in estuaries. Klotz et al (2003) notes that recreational areas, such as lakes, are traditionally not treated due to environmental and human contact concerns.

There is debate as to whether or not consumption of RIFA will kill fish. Laboratory and field studies were conducted in both Mississippi and Alabama between 1960 and 1963. Various species of deceased fish were examined and discovered to have winged sexuals in their digestive tracts and oral cavities (Crance 1965, Contreras and Labay 1999). Similarly, other ants have been found to cause distress in birds after feeding. Grant (1992) conducted an opportunistic study that observed gulls feeding on swarming *Lasius*. He noted that the gulls, upon catching the ants,
would exhibit signs of distress such as stretching their necks or regurgitation. *Lasius* possess the ability to express chemicals from their mandibular and Dufour’s glands which would certainly injure the gulls. It can be surmised that the RIFA may potentially have this effect upon consumption via fish.

It is difficult to study the effect of fire ants on fish. Even very hungry fish will not readily feed on fire ants. To overcome this behavioral avoidance, researchers force feed the ants to the fish. This method often does more damage to the fish (rupturing stomachs) than the effect of ingestion of fire ants.

Another related issue that should be considered for research is the suppression of fire ants at fish hatcheries or farms. Fish farmers, both tropical and agricultural, have great difficulty in management of RIFA populations on their property. Many do not use baits or contact insecticides due to fears over pond contamination. They are left with mound treatments that are often ineffective. If RIFA go unchecked, they have potential to torment farm employees. Farm employees typically wear shorts or go barefoot when tending ponds (Oi et al 2004), and this makes them an easy mark for the RIFA.

**Repellents**

As previously mentioned, environmental concerns present themselves when dealing with invasive ants. Natural materials are beginning to show promise in dealing with RIFA. Studies conducted by Anderson et al (2002), Chen et al (2008), Meissner and Silverman (2001, 2003), and Thorvilson and Rudd (2001) have all used natural chemicals for RIFA repellency tests. Mint oils, sage, cypress, hardwood bark, oak, juniper, and pine all showed significant repellency. Terpenoids derived from American beautyberry, Japanese beautyberry, and landscaping mulches that contained cedar also showed promise. A study that combined natural mulch materials and DEET showed promise in laboratory trials as well. However, Anderson et al (2002) notes that weathering may also have an effect on the repellent activity of the mulches, so further testing must be conducted before an absolute, finite result can be determined.

Ant behaviors, such as digging and colony formation, have been observed in regards to efficacy of repellents. Natural products such as granules with mint oil (Appel et al 2004) have been tested in laboratory and field studies. Testing proved successful; relatively low levels of mint oil were lethal and/or repellent to RIFA. However, some outdoor trials did reveal an issue with open-air testing for granules with mint oil. Appel et al (2004) noted that repellency was not detectable outdoors. Other studies focused on plants commonly found outdoors. Dr. Jian Chen’s repellency studies focus on IFA exposure to insecticides. Digging behavior, foraging behavior, exposure, and mound contact play huge roles in where and how ants are treated. Chen et al’s (2008) previous research finds successful testing with terpenoids derived from large plants. However, his 2006 study also shows that pyrethroid insecticides did not repel the ants. Some were weakened and/or killed, and the ants were halted in the digging process. The matter of concentration strength arose during these tests with high concentrations repelling the ants and lower concentrations attracting the ants.
Synthetic chemicals such as phthalates, permethrin, bifenthrin, tefluthrin, and Fluon have been mixed into sand and soil to test its effects on fire ant colonies. Chen’s 2005 study indicated that fire ant workers remove sand treated with diethyl, dimethyl, and acetone from their nests; however varying effects have been noted. Living conditions, colony size, and colony age may play a part in the effect of chemicals on ant mounds. In order for a chemical to be confirmed to be completely effective against RIFA, the results must be similar when tested on multiple colonies in multiple environmental conditions.

Soil treatment has also been pursued for nursery stock imports and exports. Both quarantine and pesticide usage laws are in place, but invasive species still manage to sneak into unsuspecting counties and countries. Oi and William’s (1996) research showed promise in treating potting soil with usage of lower levels of toxicants and specific levels of soil. Costa et al (2005) has tested plastic strips coiled beneath potted plants as a means for pest control. These strips, impregnated with permethrin, were repellent to and caused mortality of fire ants and Argentine ants.

**Depth of Fire Ant Mounds**

There are still gaps in both pre-existing and current knowledge regarding the depth of their mounds. Adams and Tschinkel (1995) conducted both field and laboratory experiments to measure nest density and competition between RIFA colonies. Their research yielded evidence of horizontal foraging tunnels reaching at least 2 m in length. Another study focused on RIFA nest morphology was conducted in both field and laboratory settings. Cassill et al (2002) made casts of outdoor nests to both determine size and take census of the nest population. This resulted in finding that chambers averaged depths of 10-80 cm below ground level.

While information can certainly be gleaned from these publications, similar research needs to be conducted in other areas where soil type and water table depth differ. This will provide a more complete picture of the structure of fire ant mounds. Not only will this knowledge satisfy the public mandate, but also it will provide us additional information that will help us understand the efficacy of mound treatments and other issues related to fire ant mounds.

**Disturbance and Insecticide Application**

Bait and contact insecticide application is a long-debated issue in pest management. Broadcast or mound treatment options are often discussed. Additionally, people may have difficulty deciding whether or not to disturb RIFA mounds before treatment. Disturbing a mound prior to treating will likely induce frenzy within the ants; they will either hide or attempt to defend the mound (Hu 2009.) When bait is applied, disturbance may delay foraging for a few minutes due to the ants recovering from disturbance vexation. However, one benefit from nest disturbance prior to baiting is that the ants will recruit more workers to collect the bait (Hu and Ding 2009.) In regards to forgoing mound disturbance, foraging activity within undisturbed colonies will start slowly but maintain consistency in foraging pace (Hu 2009.) Overall, foraging activity will still occur whether or not disturbance has taken place.
Placing baits on top of ant mounds may give PCO’s an idea of worker biomass and territory size due to foragers and be able to bait accordingly (Hu and Ding 2009, Tschinkel et al 1995.) Arguments have also been made that placing baits around the base of the mound rather than on top of the mound would be more beneficial. We currently do not know which is more effective. It is possible that the answer to this question will vary with the season, insolation, ambient temperature and humidity.

Treatment of fire ant mounds with contact insecticides encounters similar questions of whether to disturb the mound or leave it undisturbed prior to treatment. For mound treatments, it is unknown whether disturbing the mound and then treating is more effective then treating undisturbed mounds. This is compounded by conflicting information that is often found on insecticide labels. Often one insecticide label will sternly warn the user not to disturb the mound whereas another label from a competing product with the same or similar chemistry will detail how the user is to disturb the mound. It would be more effort to disturb mounds for a broadcast treatment, but this information needs to be generated. For example, a homeowner or ball field manager might cut the grass and effectively cut the tops off of all the fire ant mounds or simply flatten them by mowing. During mowing, it might be decided to treat afterward for fire ants. We need to provide the public with information about whether it is more efficacious and cost effective to treat before mowing or after.

Xing Ping Hu notes that current literature is lacking in fine-tuned methods for dealing with fire ant mounds. Her research has focused on task selection within worker ants and how bait will be distributed best within the colony. One key element to disturbance research is worker size. Hu notes that smaller ants will predominate after disturbance, while medium-sized workers will rush towards a disturbed area in means of mound defense. Hu’s main goal is to determine how combined stimuli of both disturbance and food offering will affect foraging behavior in ants. Through means of new disturbance methods, she is hoping to discover whether or not there will be a task switch. Additionally, she has extensively pursued new ways to disturb mounds due to disturbance’s ability to secure faster bait removal. One new method for disturbance includes compressing mounds via the flat end of a shovel. This will reduce the size of the mound in addition to attracting workers for bait recruitment. Holldobler and Wilson (1990, pp. 373-374) note that mound disturbance will send the ants into immediate repair mode, thus exposing them to insecticides.

**Colony identification and movement**

Questions on how to identify both RIFA mounds and individual ants are common on the eXtension.org web site. Easy-to-understand materials need to be developed and made available for the general public. These materials grant the ability to properly identify RIFA mounds and individuals in yards, other outdoor settings, and in their homes. Lack of common knowledge over RIFA mounds, particularly in newly invaded areas could lead to injury and wasted time, baits, and funds.

Colony movement in fire ants after disturbance and/or treatment has perplexed researchers and the public for many years. The problem is it is difficult to determine whether the mound has moved or if it is a new mound. Fire ants may move multiple times and/or extend
branches of an existing colony in order to better reach a food source. When new or different mounds appear after a treatment or disturbance, the landowner often assumes that the treatment did not work. At this time, there is no easy way to determine if the colony moved or it is an unseen mound that popped up. Dee Colby (pers. comm.) is currently working on a colony identification system.

**Fire Ant Identification Module**

It is clear from the Frequently Asked Questions section that the public is unclear about the identity of workers, alates (especially), and fire ant mounds. These queries may be coming from areas where there are new infestations or areas where people vacation or only reside in an area for part of the year (i.e. Florida). One method for identification would be to post pictures of fire ants at different life stages, pictures of mounds, and pictures of fire ant activities (such as swimming pools showing evidence of a mating flight.) Incorporating Cooperative Extension personnel into educational programming can also serve well. Short webisodes and/or videos via the eXtension.org site may help as well.

**Conclusion**

Clearly, a multi-state approach to addressing gaps identified is the most cost-effective and expeditious approach. Results of different research regarding effect of insecticides and depth of colonies may differ according to the soil type, soil moisture, and depth of the water table. The public has mandated that we investigate answers to these questions. It is imperative that professors, scholars, IPM officials, and other entomology experts work together to find solutions for fire ants.

Collaboration is crucial in bridging gaps in fire ant knowledge. Reaching out to other sources can lead to breakthroughs in treatment options. One professional’s bait plans can be more fully developed by scientists with granule expertise. Insect behavioral scientists can offer input to pest control companies for when and how they should treat infestations. Computing experts can assist researchers in developing user-friendly websites that allow the public to more easily access information. Additionally, researchers can publish information that would otherwise be impossible for eXtension.org personnel and the general public to access.

Fire ants are formidable challenges in our environment. Their strength as collaborative invaders is renowned. It is the duty of the human defenders to take a few cues from them; by combining intellectual strengths, we can rise to the challenge.

**References**


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Distribution of *Pseudacteon curvatus* and *Pseudacteon tricuspis* in Arkansas

Jake M. Farnum and Kelly M. Loftin
University of Arkansas

**ABSTRACT** From 1998 to 2009, *P. curvatus*, *P. tricuspis*, and *P. obtusus* were released in Arkansas. To determine *Pseudacteon* establishment and expansion, modified Puckett traps were used to monitor phorid fly species at and near 10 release sites, in counties bordering neighboring states, and along regional transects. *Pseudacteon* flies were captured in 16 Arkansas counties: Ashley, Chicot, Clark, Desha, Drew, Hempstead, Howard, Little River, Montgomery, Nevada, Perry, Phillips, Pike, Polk, Sevier, and Union. *P. curvatus* was found in areas far from release sites, suggesting dispersal from neighboring states. The range of *P. tricuspis* evidently also expanded from its initial release sites in southern Arkansas.


Monitoring of *Pseudacteon* spp. can be achieved a variety of ways: actively through direct observations of disturbed ant colonies using manual or electrical stimulation (Barr and Calixto 2005, Morrison and Porter 2005), or passively by trapping with a midden attractant and a sticky trap (Puckett et al. 2007). Until the current study, monitoring of these species had been at and near release sites to determine *P.* spp. establishment. Our objective was to determine the distribution of *P.* spp. in Arkansas through wider-scale monitoring.

**MATERIALS & METHODS**

**Trap design.** To determine presence or absence, passive trapping was used based on a modified version (Fig. 1) of a PTS (pizza tri-stand) sticky trap (Puckett et al. 2007). One trap was placed per location and retrieved 20-24 hours after placement. At time of retrieval, an 8 oz Styrofoam cup was placed over the trap, and the lid was snapped in place. The cup prevented damage and contamination to the sticky portion of the trap.

**Live ant collections.** Foraging ants were obtained by placing four plastic vials filled with 1 cm castone (Fig. 2) at locations with the Modified Puckett trap. All ants from each set of four vials from one location were placed in the same holding container; dead ants and decapitated ant heads were separated and placed into a portion cups and monitored for emerging phorid flies.

**Sampling at release sites.** Fourteen releases of *Pseudacteon* spp. were made in Arkansas from 1998 to 2009 (Table 1). Miller, Perry, Pike, and Sevier County release sites were reevaluated for this study to confirm establishment of *Pseudacteon* spp. (Fig. 3). A 1.6 km interval was used
between traps along each transect placed in Pike, Miller and Sevier Counties, and at 0.8 km intervals in Perry County. Transects were determined by locating roads and highways on aerial maps that radiated out from the release site in cardinal directions. One modified Puckett trap and four live-ant collection vials were placed at each sampling location.

**Sampling at bordering counties.** Based on established populations of *Pseudacteon* spp. in bordering counties/parishes of neighboring states: Louisiana (Henne et al. 2007), Mississippi (Thead et al. 2005), and Tennessee (Graham et al. 2003, Parkman et al. 2005, Weeks & Callcott 2008); Four modified Puckett traps were sent to each University of Arkansas Cooperative Extension Service County Agent in imported fire ant infested counties in eastern (Arkansas, Chicot, Crittenden, Desha, Lee, Phillips, St. Francis) and southern (Ashley, Columbia, Lafayette, Union) Arkansas.

**Sampling along regional transects.** Due to possible expansion of *Pseudacteon* spp. from neighboring states, sampling transects were identified and mapped in three regions: western, southeastern, and southwestern Arkansas (Fig. 4). Transects started at the state line of the adjacent state and traveled inward, using modified Puckett traps placed at 3-mile intervals and GPS coordinates recorded for each trap.

<table>
<thead>
<tr>
<th>Date</th>
<th>County</th>
<th>Species</th>
<th>Number Released</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998, July – August *</td>
<td>Drew</td>
<td><em>P. tricuspis</em></td>
<td>1,350</td>
</tr>
<tr>
<td>2002, May *</td>
<td>Pike</td>
<td><em>P. tricuspis</em></td>
<td>3,000</td>
</tr>
<tr>
<td>2002, October</td>
<td>Bradley</td>
<td><em>P. tricuspis</em></td>
<td>1,200</td>
</tr>
<tr>
<td>2003, September</td>
<td>Bradley</td>
<td><em>P. tricuspis</em></td>
<td>1,300</td>
</tr>
<tr>
<td>2004, May *</td>
<td>Miller</td>
<td><em>P. tricuspis</em></td>
<td>2,500</td>
</tr>
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<td>Sevier</td>
<td><em>P. tricuspis</em></td>
<td>4,200</td>
</tr>
<tr>
<td>2005, October</td>
<td>Clark</td>
<td><em>P. curvatus</em></td>
<td>8,500 *</td>
</tr>
<tr>
<td>2006, September</td>
<td>Perry</td>
<td><em>P. curvatus</em></td>
<td>15,816 *</td>
</tr>
<tr>
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<td>Perry</td>
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<td>2008, June</td>
<td>Jefferson</td>
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<tr>
<td>2006, October</td>
<td>Polk</td>
<td><em>P. obscurus</em></td>
<td>3,200 *</td>
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<td>Grant</td>
<td><em>P. curvatus</em></td>
<td>22,286 *</td>
</tr>
<tr>
<td>2009, June</td>
<td>Jefferson</td>
<td><em>P. tricuspis</em></td>
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<tr>
<td>2009, September</td>
<td>Garland</td>
<td><em>P. tricuspis</em></td>
<td>360</td>
</tr>
</tbody>
</table>

* Numbers released based on 35.3 g S. invicta workers, 800 g/m² and 30% parasitism
  * Numbers released based on 65.9 g S. invicta workers, 800 g/m² and 30% parasitism
  * Numbers released based on 23.7 g S. invicta workers, 450 g/m² and 30% parasitism
  * Numbers released based on 105.4 g S. invicta workers, 300 g/m² and 30% parasitism

Table 1. Release dates, locations and *Pseudacteon* spp. in Arkansas
Figure 1. Modified Puckett trap

Figure 2. Live ant collection vial
RESULTS

Live ant collections. Approximately 22,000 live ants from 73 locations along transects from three release sites (Miller, Perry, and Sevier Counties), and in Hempstead County were collected. After holding ants for 7 weeks, only 3 ant heads contained a puparium, and no flies emerged.

Release sites. Of the 20 traps retrieved from Sevier County, no *Pseudacteon* spp. were caught. Traps from Miller, Perry, and Pike Counties captured *Pseudacteon* spp. Along the Miller County release site transects, *P. curvatus* was captured at three locations north of Texarkana, in the
southeastern part of Little River County (Fig. 5). However, the phorid fly release in Miller County in 2004 was of *P. tricuspis* (Fig. 6 E).

**Bordering counties.** Puckett traps from Columbia, Lafayette, and St. Francis Counties were void of *Pseudacteon* spp., although all traps returned from Phillips County on the MS border captured *P. curvatus*. Phillips County is the only county in Arkansas to have only *S. richteri* or its hybrid and no record of *S. invicta*. Sampling locations in Phillips Co. were located on the western levee of the Mississippi River, northwest of Friars Point, MS (Fig. 5 D), adjacent to counties in Mississippi and Tennessee with known *S. richteri* populations (Streett et al. 2006, Oliver et al. 2009).

**Regional transects.** A total of 176 traps were placed along transects in western, southeastern, and southwestern Arkansas (Fig. 4).

**Western:** 28 traps contained *P. curvatus*, and 2 *P. tricuspis*. All four transects in this region included captures of *P. curvatus* (Fig. 5).

**Southeastern:** *P. curvatus* was found at one location in Union County, north of El Dorado, and on 12 traps along each of the other transects (SE2 and SE3) to the east (Fig. 5).

**Southwestern:** *P. tricuspis* was captured at two locations along a transect (SW3) of the southwestern region of Arkansas (Fig. 6).

![Figure 5](image)

**Figure 5.** *Pseudacteon curvatus* release sites in (A) Clark Co., (B) Grant Co., (C) Perry Co., and capture locations including (D) Phillips Co., Arkansas.
CONCLUSION

While sampling live ants was of little value, the most efficient method was using a passive trap like the modified Puckett trap. Because it is deployed quickly, multiple traps can be placed over a large area which allows continuous and simultaneous sampling (Puckett et al. 2007). The addition of the protective cup to the original design allowed longer storage time between collection and examination of the trap, protection of the sticky portion, and reduced contamination.

It is unknown if the modification of the trap design and placement (on a disturbed mound with live ants versus Puckett traps placed in an open area with midden (Puckett et al. 2007)) had any influence on *Pseudacteon* spp. captured.

The results suggest establishment and expansion of *P. curvatus* from the release site in Perry County, and *P. tricuspis* from the release site in Pike County. While limited, the current range of *P. tricuspis* in Arkansas appeared to be along a narrow 86 km band stretching from northwest Pike County to south central Nevada County, and 25 km west of the release site (Fig. 6). The current distribution of *P. curvatus* in Arkansas suggested natural movement from surrounding states.

Despite extensive sampling across southern Arkansas, many areas remained unsampled. Additional trapping would provide a better understanding of the distribution of *Pseudacteon* spp. in Arkansas.
ACKNOWLEDGEMENTS  Thanks to Anne-Marie Callcott of the USDA APHIS Gulfport, MS lab. Amy Bass, Amy Croft, & Deborah Roberts of the Florida DACS for supplying phorid flies. Ed Brown, Jerry Clemons, Randy Forst, Rex Herring, Mike McCarter, Shawn Payne, Doug Petty, Amy Simpson, Rebecca Thomas, Shaun Rhodes, Carla Vaught, Joe Vestal, & Danny Walker of the University of Arkansas Cooperative Extension for placing traps. Michael Hamilton & Robert Goodson for collecting imported fire ants for identification. Joel Bard of the USDA APHIS for his continued support of this project. Ricky Corder of the U of A Cooperative Extension Service.

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Impact of Methoprene and Pyriproxyfen on *Pseudacteon tricuspis* (Diptera: Phoridae), a Parasitoid of the Red Imported Fire Ant (Hymenoptera: Formicidae)

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**ABSTRACT** The impacts on *Pseudacteon* spp. by insecticides, particularly insect growth regulators, used in controlling imported fire ants have yet to be explored. Red imported fire ants parasitized by *Pseudacteon tricuspis* were exposed to methoprene and pyriproxyfen, the active ingredients used in some fire ant baits. This experiment tested the effects of the two IGRs on the phorid fly larva at two time intervals (days 6 & 10 post-parasitism) when the larva was present in the thorax and head of the adult ant host, respectively. The mean proportion of emerged *P. tricuspis* from methoprene and pyriproxyfen treatments was significantly reduced relative to the control. Timing of exposure to the IGRs (days post-parasitism) did not have a significant impact on the emergence of the phorid fly.

**INTRODUCTION** There are two main forms of baits: toxins which kill all castes within the colony, and insect growth regulators (IGRs) which target the reproductive (queen) capabilities of the colony, with no acute effect on the sterile female worker ants (Banks 1986). Methoprene and pyriproxyfen are among the most commonly used IGRs for imported fire ant control (Oi and Drees 2009).

In 1995, phorid flies of the *Pseudacteon* genus were imported as classical biological control agents against the red imported fire ant. Of the 18 species known to attack ants of the *Solenopsis saevissima* complex in South America (Porter and Pesquero 2001, Folgarait et al. 2005), four species have been released in the U.S.: *P. litoralis, P. curvatus, P. tricuspis*, and *P. obtusus*. Interactions of IGRs and parasitoids have been documented in many species, although no reports were found on the impact of IGRs on *Pseudacteon* spp. The objective of this study was to determine the potential impact of methoprene and pyriproxyfen on developing *P. tricuspis* larvae.

**MATERIALS & METHODS** Ants parasitized by *P. tricuspis* were obtained from the Florida Department of Agriculture and Consumer Services, Biological Control Rearing Facility in Gainesville, FL. Parasitized ants were dissected to determine the developmental stage and location of the parasitoid within the host. Based on this information, day 6 and 10 post-parasitization was chosen to insure ant exposure to IGRs when *P. tricuspis* larvae were found in the thorax and head, respectively. Technical grade pyriproxyfen and methoprene, obtained from Chem Service, Inc. (West Chester, PA) were mixed into solution with 99.5% acetone. Based on their percent purity, calculations were made to produce a rate (4,940 µg/ml) comparable to the amount active ingredient in 1 g of formulated bait (5,000 µg). Tests were conducted with the two chemicals at days 6 and day 10 post-parasitization.

Four replications were evaluated from June 2009 to August 2009. Parasitized ants, obtained from the Florida lab were divided into 6 treatment groups, 400 ants each: ants exposed to pyriproxyfen on days 6 (P6) and 10 (P10), ants exposed to methoprene on days 6 (M6) and 10 (M10), and ants exposed to acetone control on days 6 (C6) and 10 (C10). On the day of
treatment, 1 ml of 4,940 µg/ml solution was applied to a 9-cm diameter filter paper (Fig. 1). Ants were exposed to the treated filter paper for 6 hours (Fig. 2). Upon reintroduction to the holding container, ants were provided a new sugar source and water tube. Immediately following the 6 hour exposure, dead ants were removed, and were removed daily from all treatments. Dead ants were counted and ant heads separated and placed into individual portion cups (Fig. 3).

Due to differing number of ants exposed at day 6 and day 10, the number of ants exposed to each treatment, number of decapitated heads, and number of emerged flies were standardized into proportions. Data were analyzed using factorial analysis of variance (ANOVA), and multiple comparisons made using a protected LSD test.

Figure 1. Technical grade methoprene & pyriproxyfen solutions applied to filter paper

Figure 2. Exposure to IGRs via treated filter papers

Figure 3. Midden and ant heads placed into portion cups
RESULTS The mean proportion of exposed ants that were decapitated varied significantly across the time and treatment interaction (Fig. 4). There was no significant differences in the proportion of decapitated ants among any of the day 6 treatments. Furthermore, no significant differences in decapitation between the methoprene treatments at the two time intervals or between the two pyriproxyfen treatments were observed. Ants exposed to methoprene at day 10 had the lowest proportion of heads decapitated (0.18), relative to the pyriproxyfen at day 10 (0.25) and control at day 10 (0.28).

Figure 4. Mean proportion ± SE of decapitated heads from ants exposed to IGRs. Bars with the same letter label are not significantly different (P > 0.05), Protected LSD, p-value from AOV = 0.0045. Treatments are abbreviated as follows (P6, pyriproxyfen at day 6; M6, methoprene at day 6; C6, control at day 6; P10, pyriproxyfen at day 10; M10, methoprene at day 10; C10, control at day 10).

All treatments significantly reduced fly emergence from decapitated heads relative to controls (Fig. 5). There was no difference in emergence between the times of exposure or between the two IGR treatments. It is worth noting that the methoprene treatment at day 10 had no flies emerge, and the pyriproxyfen treatment at day 10 only had one fly emerge (0.002) from all four replications. Emergence from IGR treatments were significantly less than that of controls with 19 flies emerging from the pyriproxyfen treatment (0.032), and 13 flies from the methoprene treatment (0.012), versus 190 flies from the control (0.256).
There was no significant difference in the mean proportion of flies emerged from exposed ants between the times of exposure or between the two IGRs (Fig. 6). Overall, the proportions of emerged flies from ants exposed were significantly greater in the controls than the treatment groups. These data suggest methoprene and pyriproxyfen reduce emergence of *P. tricuspis*.

**DISCUSSION** Because the third instar decapitates the ant’s head prior to pupation (Porter 1998, Consoli et al. 2001), decapitation was deemed an observation of importance, not the formation of the puparium. Decapitation was a visual indicator of the status of the larva inside the ant, having survived to third instar. Furthermore, if the two treatments were to have had an impact on decapitation, it suggests an impact of the IGRs on previous instars. There is less than
10% difference between the most disparate treatments (Fig. 4), which suggests little biological difference, even though there are statistical differences among treatments.

Methoprene and pyriproxyfen are not only used in imported fire ant suppression, but also used extensively to control Dipteran pests. These products induce mortality of the last instar, a reduction in emerging adults, and an inhibition of development. Although fly larvae were exposed to IGRs via cutaneous absorption by the ant, similar reduction of development were seen.

Feeding-stage larval parasitoids are more sensitive to IGR exposure, due to the consumption of host tissue and hemolymph (Beckage 1985). Our data suggests the impact on development occurred after decapitation of the ant’s head.

ACKNOWLEDGEMENTS  Thanks to Deborah Roberts, Amy Croft, and Amy Bass, Florida DACS in Gainesville, FL for supplies used in the release and parasitization of the ants; Anne-Maria Callcott, USDA-APHIS, Gulfport, MS for technical assistance; and Ricky Corder of the U of A Cooperative Extension Service.

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RIFA Effects on Some Native Ants in Arkansas Prairies

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Abstract

It has been suggested that Red Imported Fire Ants (Solenopsis invicta) [RIFA] negatively influence native ants where it has colonized. Most studies showing these negative effects have followed invasion of new lands as RIFA have increased their range. However, others have suggested that RIFA do not impact native ants that much. Their evidence comes from studies conducted many years after RIFA have colonized an area, and show that native species are at almost pre-infestation levels. Our objective was to identify RIFA influences in space, sampling ant communities concurrently in infested and uninfested areas. To do this, ants were sampled in selected prairies in extreme southern Arkansas where RIFA has been part of the ant community for more than 30 years, and in uninfested prairies in east-central Arkansas where RIFA range expansion is heading. Infested prairies included Grandview Prairie Wildlife Management Area, Warren Prairie Natural Area, and Locust Ridge Prairie (on Felsenthal National Wildlife Refuge). Uninfested prairies included 4 Arkansas Natural Heritage Commission Natural Areas: Downs Prairie, Konecny Prairie, Railroad Prairie, and Roth Prairie. Ants were sampled using pitfall traps, distributed along 3 20-pitfall traplines per prairie, and collected 3 to 4 times over the summer at about 1-month intervals. Ant species lists by prairie were assembled using the quantitative measure total number of pitfalls that collected a species over the entire summer. This count measured the number of times a species was collected, a measure of relative abundance. To analyze ant communities over all sites, multivariate statistics, specifically hierarchical cluster analysis and indicator species analysis (ISA), were used. Cluster analysis identified the overall differences in prairie communities and ISA identified specific species significantly affected by prairie location. Cluster analysis classified the infested and uninfested prairies into 2 distinct groups; showing that location was important. Of course, it is surmised that RIFA induced this location effect. In fact, RIFA was one of 3 species identified by ISA as being significantly affected by prairie location. RIFA was absent from the east-central prairies, but it made up more than 78% of the ant community on each of the 3 infested prairies. The other significantly affected species were Pheidole dentata and Temnothorax pergandei, both absent in RIFA infested prairies. Pheidole dentata was the dominant ant in the east-central uninfested prairies. So, RIFA seem to affect individual species rather than the entire community as a whole.
A Characterization of mtDNA Haplotypes for Two Imported Fire Ant Species and Their Hybrid

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INTRODUCTION

Since their introductions in the early 20th century, the two imported fire ant species have rapidly expanded their U.S. ranges. One, then the other species radiated through large parts of the southeastern states. The red imported fire ant, *Solenopsis invicta*, supplanted much of the earlier, black imported fire ant (*S. richteri*), arrival’s territory and occurs in much of the southeastern states including the most of the southern and central parts of TX. *Solenopsis invicta* has more recently become established in VA, CA and NM (Fig. 1).

Fig. 1. USDA-APHIS-PPQ Imported fire ant quarantine map.

A fertile, hybrid form of the two species, *Solenopsis invicta x richteri*, can generally be found from the northern half of MS and AL, to eastern TN and northwestern GA.

Failures to limit the spread of imported fire ants through chemical control have increased the focus on alternative methods, particularly through biological control. Parasitoids and
entomopathogens often show species specificity, increasing the need for proper identification of these ants to species or hybrid form.

Identification using morphological characters can be inexact, especially for the hybrid imported fire ant. Gas chromatography and mass spectrometry (GC/MS) analyses (Menzel et al. 2008) work very reliably, but access to the necessary apparatus may be limiting. Molecular testing provides a relatively inexpensive set of tools to assist in the identification process.

Ross and Shoemaker (1997) described the use of random fragment length polymorphisms (RFLP’s), of a COI - COII region of mitochondrial DNA, (mtDNA), for use with $S. \text{invicta}$. Recently, Jacobson et al. (2006) used RFLP technique on an associated mtDNA sequence to distinguish $S. \text{invicta}$ from $S. \text{xyloni}$.

Here we add an additional enzyme, SspI, to a subset of Ross and Shoemaker’s and apply this method across both imported fire ant species and their hybrid. This application provides a means for delineating several mtDNA haplotypes and has the potential for aiding species identification and for measuring population dynamics.

MATERIALS AND METHODS

**Collections:** Ants from MS, AR, TN, AL, CA, GA, SC, and FL were collected in ethanol for DNA isolation and testing. More concentrated collections were made at sites in MS and AL and a known $S. \text{richteri}$ zone in western TN. Workers from over 140 colonies were collected, as well as 51 newly mated queens and 12 sperm samples from the queens’ spermathecae. Ants from colonies were also collected in hexane as described by Menzel et al. for cuticular hydrocarbon and venom alkaloid analyses. The GC/MS procedures were performed at the USDA-ARS facility in Stoneville, MS. Those whose species / hybrid status that was not verified through GC/MS techniques had other previously proven molecular tests performed to aid in that purpose.

**mtDNA markers:** Using PCR, we amplified an approximately 4 kb sized portion from the COI-COII mtDNA region. We first selected seven restriction enzymes (New England Biolabs, Ipswich, MA) to perform digestions on the PCR product. After the initial screening, two enzymes, ($Hae$III and $Mlu$I) showed low variability in number of cut sites and we stopped their use. We chose five, ($Msp$I, $Taq$αI, $Ssp$I, $Bam$HI, and $Hin$fI), that showed digestion patterns on agarose gels indicative of promising levels of polymorphism. Samples were electrophoresed on two percent agarose gels in SB buffer and stained with GelRed DNA stain (Biotium Inc., Hayward, CA) for imaging under UV light.

RESULTS

We assigned letter codes representing the scored digestion patterns present on agarose gels, (see Fig.s 2-4). Some of the restriction enzymes, particularly $Taq$αI and $Bam$HI, indicated the presence of higher polymorphism than others.
Digestion pattern examples

**Fig. 2.** Digested mtDNA electrophoresed on agarose gel. M=Marker. Enzyme used = \textit{SspI}.

**Fig. 3.** Digested mtDNA electrophoresed on agarose gel. M=Marker. Enzyme used = \textit{TaqαI}.

### Restriction Enzymes

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>\textit{SspI}</th>
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**Fig. 4.** Haplotype designation based on digested mtDNA patterns for five restriction enzymes.

- Only a single, unique mtDNA haplotype, \textbf{I}, was indicated for \textit{S. richteri}, contrasting with five for \textit{S. invicta}, (Fig. 4). Haplotype \textbf{I} was not found in \textit{S. invicta} samples and types \textbf{II} – \textbf{VI} were absent in \textit{S. richteri} samples.
- Hybrid imported fire ant samples collectively exhibited the \textit{S. richteri} haplotype and all the \textit{S. invicta} haplotypes, with the notable exception of type \textbf{VI} (thus far, found only in \textit{S. invicta} samples).
- Haplotype I was not found in the southern half of MS or AL, and rarely were any hybrid fire ants detected in this area.
- Reproducibility: We included 31 additional samples collected from the same monogyne colonies to check for consistent results. The same haplotype was indicated in all cases.

Frequency of mtDNA haplotypes across all samples

![Graph showing the frequency of mtDNA haplotypes](image)

**Fig. 5.** Total number of mounds, queens and sperm mtDNA samples producing each mtDNA haplotype were included in counts to measure frequency. *Replicates within monogyne colonies were excluded.

- Even rarely occurring haplotypes, (IV-VI), were found at considerable distances from site to site (see Figs. 5 and 6).
CONCLUSIONS

- The use of BamHI digest is critical for our haplotype delineation. This enzyme, when paired with MspI, TaqαI or HinfI will distinguish the haplotypes described here.
- New tests for species types: The RFLP test using a single enzyme, i.e. the SspI, BamHI or TaqαI enzyme alone, could be used as a test delineating the *S. richteri* haplotype from *S. invicta* type DNA.
- This RFLP distinguished five apparent haplotypes within *S. invicta*, versus four found by Ross and Shoemaker (1997). It was also shown capable of illustrating a unique haplotype of *S. richteri*.
- The hybrid fire ant’s inheritance of haplotypes originating from either of the species is in agreement with the theory that there is no absolute bias of mating between the species, (i.e. female *S. invicta* never mating with male *S. richteri*). Consequently, precise identification of the hybrid form through molecular means is possible, (a greater mix of markers from both species may be present). Alternatively, absolute accuracy in identifying an ant of one of the pure species requires the use of some other method.

What’s next?

- We are evaluating the development of SNP, (single nucleotide polymorphism), tests that would use critical polymorphic sequence regions to evaluate haplotypes through a single PCR step.
- Further sampling is being done to check for type VI haplotype in hybrid imported fire ants and for type I away from *S. richteri* or accepted hybrid zones. We would expect haplotype VI to be eventually found in hybrid fire ants as there has yet to be an exclusion of either a nuclear or a mitochondrial marker in previous studies.
ACKNOWLEDGMENTS

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Worker Ant Foraging Response On And Near Mounds Of The Red Imported Fire Ant

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Abstract

Red imported fire ants are an invasive species that has infested over 300 million acres in the southern United States. Management of this insect pest often relies on the use of food lures and granular bait insecticides. This trial was conducted to determine the response of the red imported fire ant workers when a food lure was placed on the center of the nest and then 1 foot, 3 feet, and 6 feet from the nest. Results demonstrate that red imported fire ants forage on top of their mounds or colonies. These data should help convince insecticide manufacturers to allow placement of the granular baits directly on top of undisturbed mounds in addition to scattering the bait around the mound.
Arginine Kinase: Differentiation of Gene Expression And Protein Activity in The Red Imported Fire Ant, *Solenopsis Invicta*

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Abstract

Arginine kinase (AK), a primary enzyme in cell metabolism and adenosine 5’-triphosphate (ATP) -consuming processes, plays an important role in cellular energy metabolism and maintaining constant ATP levels in invertebrate cells. In order to identify genes that are differentially expressed between larvae and adults, queens and workers, and female alates (winged) and queens (wingless), AK cDNA was obtained from the red imported fire ant. The cDNA sequence of the gene has open reading frames of 1065 nucleotides, encoding a protein of 355 amino acid residues that includes the substrate recognition region, the signature sequence pattern of ATP: guanidino kinases, and an "actinin-type" actin binding domain. Northern blot analysis and protein activity analysis demonstrated that the expression of the AK gene and its protein activity were developmentally, caste specifically, and tissue specifically regulated in red imported fire ants with a descending order of worker > alate (winged adult) female > alate (winged adult) male > larvae > worker pupae ≈ alate pupae. These results suggest a different demand for energy-consumption and production in the different castes of the red imported fire ant, which may be linked to their different missions and physiological activities in the colonies. The highest level of the AK gene expression and activity was identified in head tissue of both female alates and workers and thorax tissue of workers, followed by thorax tissue of female alates and abdomen tissue of male alates, suggesting the main tissues or cells in these body parts, such as brain, neurons and muscles, which have been identified as the major tissues and/or cells that display high and variable rates of energy turnover in other organisms, play a key role in energy production and its utilization in the fire ant. In contrast, in the male alate, the highest AK expression and activity were found in the abdomen, suggesting that here energy demand may relate to sperm formation and reproduction.

References

Using Fire Ant Demonstrations to Open the School IPM Door

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Abstract. In 2009, the University of Tennessee Urban IPM Lab, in cooperation with UT Extension agents, undertook another strategy to increase integrated pest management (IPM) adoption in Tennessee’s schools. To “get our foot in the door” in regards to increasing IPM adoption, fire ant management demonstrations were conducted on the grounds of at least one school in three school systems located in different Department of Education regions (Southeast, Upper Cumberland and South Central). Fire ants are a serious pest around schools: they are a medical concern due to their stings, can disrupt the learning environment and can interfere with electrical equipment. Ants/fire ants were the third most frequent pest reported and tied for second in the most troublesome category in the 2002 UT school pest management survey. Conducting the fire ant management demonstration prior to the indoor IPM program allowed us to demonstrate our commitment to managing and reducing risk from pests and pesticides at schools and establish a rapport with the school personnel, and a provided a segue into an indoor IPM program. We intend to use the 2009 demonstration schools as IPM models for surrounding school systems in 2010 and subsequent years.

Issue. School IPM programs aim to reduce and balance risks from pests and pesticides to school occupants and the environment. Children spend considerable time at school and therefore increase their risk of pesticide exposure if pesticides have been applied in a manner inconstant with IPM. Pests pose risks from venomous bites, disease transmission, allergic responses, equipment damage, and may disrupt the learning environment. IPM should achieve long term, environmentally sound pest suppression using a wide variety of technological and management practices. Control strategies in a child-serving facility IPM program extend beyond the application of pesticides to include structural, habitat and procedural modifications that reduce food, water, harborage, and access used by pests (http://schoolipm.ifas.ufl.edu/).

Justification. Why rural schools? In 2002, a school pest management survey mailed to all school districts in Tennessee had a 36% response rate. Although 63% of respondents were from rural areas, IPM was only used in 18% of the rural districts. We have created awareness of school IPM with just about every stakeholder group involved with pest management in schools, but personnel from rural schools have been among the most difficult to reach. Limited budgets (including travel budgets for meetings), personnel and time to deal with pest management make IPM adoption a challenge in rural areas. With this demonstration approach we have brought a school IPM program to the rural schools. Fire ants are a serious pest around schools: they are a medical concern due to their stings, can disrupt the learning environment and can interfere with electrical equipment. The “green” approach is used as an incentive for schools in urban areas to...
adopt IPM, and providing fire ant management advice may get rural schools interested in and involved with IPM.

Tennessee school pest management surveys conducted in 1997, 2002, and 2008 (http://eppserver.ag.utk.edu/School%20IPM/sch_ipm.htm) indicated that slow, but steady, progress is being made towards adoption of school IPM. In 1997, indoor school IPM adoption was estimated at 12% (74% return) and in 2002, had reached 25% (36% return). In 2008, although only 6.7% of school districts completed the survey, 54% of schools used high IPM. It appears the rate of IPM adoption is doubling about every 5 years. With continued effort, we hope to have all schools using IPM by 2013.

**Objective.** This poster presents efforts to achieve one objective of our 2009 USDA Extension IPM Grant which has one of its ultimate goals of getting all Tennessee’s schools using IPM. This one objective is to increase school IPM adoption in rural areas through (a) outdoor fire ant and (b) indoor IPM demonstrations.

**Materials and Methods (a).** Extension agents were contacted in Rhea, Cumberland and Moore Counties in the TN Department of Education rural school regions of Southeast, Upper Cumberland and South Central, respectively. Extension agents are expected to become the local pest management trouble-shooting experts in their counties. Either we or the county agents contacted each school system’s facilities manager and inquired about schools with fire ants

![Broadcasting fire ant bait.](image)

Figure 1. Broadcasting fire ant bait.

Fire ant baits were either applied as a broadcast application (Fig.1) or an individual mound treatment (IMT). Fire ant bait was applied as listed in the figure captions for Cumberland County (Figs.3-5), Moore County (Fig.6) and Rhea County schools (Figs.7-10). Mound activity was
monitored by us or the agents at approximate two week intervals. At 6 weeks, all active mounds were treated with Amdro (hydramethylnon) because school would be starting shortly thereafter (Fig. 2).

![Image](image.png)

**Figure 2.** Bait individual mound treatment (IMT).

**Results and Discussion(a).** While the Advion individual mound treatments were very effective at all schools (Figs. 6, 11 and 12), these fire ant demonstrations revealed the problem encountered with individual mound treatments. Mounds, typically smaller or flatter, are overlooked and not seen until after a rain or until they have grown larger and, thus, miss treatment. Broadcast applications of IGR (Extinguish, Distance), combination IGR/metabolic inhibitor (Extinguish Plus) or sodium-channel blocker (Advion) bait were slower than IMTs. However, past experience indicates that IGRs provide more long-term control by reducing the ability of newly mated queens to establish in these areas because workers are not killed and defend their territory.

When fire ant mounds are abundant, our recommendation is to broadcast a fire ant bait (IGR, or IGR/metabolic inhibitor or other) twice a year. With experience from this demonstration and thresholds developed in the eXtension web site, [http://www.extension.org/pages/School_IPM_Action_Plan_for_Fire_Ants](http://www.extension.org/pages/School_IPM_Action_Plan_for_Fire_Ants), a new publication describing fire ant treatment around schools will be developed.

One other problem was encountered with the fire ant treatments. As we left flags where fire ant mounds were found, school personnel were treating mounds around the schools. While we are encouraged that school personnel were scouting and treating for fire ants, this may have affected our results. Our treatments should have been noted in the school IPM log book, but some school personnel had not been informed of the manual and were unaware of our treatments. Also, because we often treated mounds late in the day, records of these applications were emailed to appropriate individuals. But, these may not have been placed in the log book. This further emphasizes the importance of communication. All parties potentially involved with pest management should be educated and trained prior to any application. This encourages us to hold fire ant specific training for school personnel and others this winter. Most school personnel
applying fire ant control products had not been trained in fire ant management or pesticide use. Tennessee law requires persons applying pesticides inside schools to be under the direct supervision of a licensed applicator, but this does not apply to outdoor applications.

**Figure 3.** Homestead Elementary of Cumberland County Schools. All mounds individually treated with Advion.

**Figure 4.** Crab Orchard Elementary of Cumberland County Schools. All mounds individually treated with Advion.
Figure 5. Stone Memorial High School of Cumberland County Schools. All mounds individually treated with Advion.

Figure 6. Moore County High School. All mounds individually treated with Advion. Two weeks after treatment, only one mound out of 34 was still active (97.1% reduction).
Figure 7. Rhea County High School. Mounds 1-35 on incline between practice and football field individually treated with Advion; 36-54 broadcasted with Distance; 55-63 broadcasted with Extinguish Plus; 93-104 along southern fence of football field broadcasted with Advion; all other mounds individually treated with Advion.

Figure 8. Spring City Middle School of Rhea County Schools. All mounds individually treated with Advion.
Figure 9. Rhea Central Elementary. All mounds individually treated with Advion.

Figure 10. Graysville Elementary of Rhea County Schools. Mounds in purple rectangle broadcasted with Extinguish in 14 ft band around swing set in playground; all other mounds individually treated with Extinguish.
Figure 11. Percentage reduction and mound number of imported fire ants using all original mounds (triangles) and new mounds only (squares) individually treated with Advion in Cumberland County.

Figure 12. Percentage reduction in fire ant mound number at Rhea County. This includes the original mounds and the new mounds. Reductions pertaining to original mounds only would have been higher.
Materials and Methods (b). School IPM workshops were conducted in Cumberland and Moore Counties in August of this year. We are still attempting to conduct a meeting in the Rhea County school system.

In the workshops, we described school IDM to kitchen, maintenance and custodial staff in these two school systems and discussed their roles.
To provide hands-on appreciation of the program, all attendees were given an IPM inspection sheet and an inspection was conducted of the high school cafeteria and perimeter.

A representative of each school was provided school IPM logbooks and asked to place these in the front office with a secretary.

<table>
<thead>
<tr>
<th>Number of attendees at all school IPM workshops</th>
<th>Mean pre-training quiz scores</th>
<th>Mean post-training quiz scores</th>
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<tr>
<td>49</td>
<td>61%</td>
<td>82%</td>
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</table>
Results from a pre- and post-training quiz for all school IPM workshop attendees for all schools combined indicated a 21% increase in test scores or knowledge of IPM.

An inspection kit consisting of a flashlight, screwdrivers, telescoping mirror, spatulas, forceps, markers, zip-top bags, hand lens and glue boards was given to each school system to assist in their adoption of IPM.

In October, the first complete IPM inspection was conducted at a high school in each system with the pest management professional, a potential local technical IPM coordinator, a county agent, our staff and other school personnel. An inspection score for each site was calculated out of 500 points. We will alternate monthly inspections with monthly phone calls to keep appraised of the IPM in each school.

**Results and discussion (b).** Scores from the initial inspections reflected the age of the school. The older schools needed more structural repairs to reduce conducive conditions. The demonstration schools are well on their way to using high level IPM. In most cases, logbooks are being completed correctly, repairs are planned or have occurred, and communication is improving. We plan to use these schools as models for surrounding school systems and, if approved for future funding, will invite staff from surrounding school systems to observe IPM in action.

Partial support for this program was provided by an Extension IPM Grant.
Degradation of IFA Program Chemicals in Various Potting Media

Lee McAnally, Anne-Marie Callcott, Connie Ramos USDA, APHIS, PPQ, CPHST Lab, Gulfport, MS

INTRODUCTION

For certification in the Federal Imported Fire Ant Quarantine (7CFR 301.81), containerized nursery stock can be treated by incorporating granular insecticides into the potting media prior to potting or by drenching with insecticidal solutions prior to shipment. Various initial treatment dose rates result in various certification periods (see tables below).

* If all other provisions of Fire Ant Free Nursery Program are met

For quality assurance, to determine whether the nursery properly applied the insecticide to the potting media, PPQ and state inspectors routinely collect media samples which are submitted to laboratories for chemical analysis to determine amount of insecticide present in the media (usually reported in parts per million – ppm). These media samples can be collected from nurseries using the quarantine treatment, as well as from nursery container shipments with suspect or confirmed IFA infestations.

Trials to determine effective dose rates and certification periods of insecticide treatments focused on the efficacy of the insecticide on the target insect, and no studies were conducted to determine the chemical degradation of the insecticide in potting media. In late 2004, a series of trials were initiated to determine levels of program chemicals detected by chemical analysis over the certification/aging period of the treated media. This testing was done in cooperation with the CPHST Gulfport Lab Chemical Analysis section who conducted the chemical residue analyses. Data collected from these trials will allow the quarantine program to better evaluate results from chemical analyses of samples collected by inspectors.

The initial test dealt with incorporated treatments and was prematurely terminated due to hurricane Katrina. The data generated by the limited sampling was inconsistent and highly variable, and no significant conclusions could be formed with this data. As a result, a new
incorporated treatment trial was initiated in 2006 incorporating lessons learned about the sampling and mixing procedures. Further tests of drench applications were conducted in 2008 and 2009.

MATERIALS AND METHODS

Incorporated Treatments

Potting media used in this test were: MAFES media (3:1:1 pine bark: sphagnum peat moss: sand with bulk density = 875 lb/cu yd); Windmill media (Windmill Nursery, Folsom, LA with bulk density = 310 pounds per cubic yd).

The MAFES media is being tested in two ways; bulk mixing where the individual components of the untreated media was premixed in a large quantity, measured into 1.5 cu. ft. loads and then chemical treatments applied, and batch mixing where each individual component was measured in the correct proportions for one 1.5 cu. ft. mixer load and chemical treatment added at the same time (Fig. 1). The same amount of chemical was added based on the single premixed bulk density of the MAFES media. Windmill media is obtained in bulk and required no difference in handling.

To insure consistency over the quarantine all incorporation applications are made based on the dry weight bulk density of the media. However, the question on efficacy of bifenthrin and/or adsorption of bifenthrin to the media when mixed in thoroughly saturated media or very dry media has been raised. Each media/mixture type was then mixed either wet or dry at 10 and 25 ppm. Dry mixing meant that no additional moisture other than what was already in the media was added. The wet mixes were done by adding approximately 1 liter of water per mixer load (1.5 cu. ft.). The wet loads were allowed to mix for approximately 5 minutes to ensure a uniform moisture content before the chemical treatments were added. A portable cement mixer (2 cu ft capacity) was used to blend the chemical into the potting media, and was operated for 15 minutes per load to insure thorough blending. Treated media was then placed into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions (Fig. 2). A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week.

Fig. 1 Mixer load being dumped in preparation for potting

Fig. 2 Pots with treated media exposed to the weather
Drench Treatments

Drench trials were conducted in 2008 and 2009. The first trial in 2008 sought to identify the effect of pre-treatment moisture levels in the potting media on the efficacy and/or adsorption of chlorpyrifos and bifenthrin. Therefore, the media were tested in two ways; application to media that was watered to saturation prior to treatment and application to dry media.

The bifenthrin treatments were made using both a 2EC and a flowable formulation of the product while chlorpyrifos treatments were made using a 4EC formulation. Both bifenthrin formulations were applied at a theoretical dose rate of 25 ppm while chlorpyrifos was applied at a rate equivalent to 4 fl. oz. 4EC/100 gallons of water (ca. 124 ppm in Windmill media and 56 ppm in MAFES media). The drench solutions were applied to standard 1-gallon nursery pots at a rate of 1/5 the volume of the container (ca. 450ml drench solution) as called for in the quarantine manual (Fig. 3). The pots were weathered as mentioned above.

The second drench trial conducted in 2009 evaluated only bifenthrin. The first trial raised the question of whether each individual pot received the same initial dose rate and therefore did not accurately reflect the rate of degradation over time. As a result, the drench solutions where applied to standard 5-gallon nursery pots at a rate of 1/5 the volume of the container (ca. 1 gallon drench solution) as called for in the quarantine manual (Fig. 4). Three pots were treated for each treatment type with each individual pot being considered a replicate. The treatments were made using both a 2EC and a flowable formulation of bifenthrin. Both formulations were applied at a theoretical dose rate of 10 ppm and 25 ppm.

Sample Collection

Immediately after treatment, samples were taken for chemical analysis. For the incorporated trial and the first drench trial each sample consisted of one full pot and three such samples (replicates) per treatment were submitted for analysis. For the second drench trial each sample consisted of a core sample from each 5-gallon pot (replicate) with three such samples per treatment were submitted for analysis.
For the incorporated trials samples were taken at 0, 3, and 6 months post-treatment for the 10 ppm treatments and at 0, 6, 12, 18 and 24 months post-treatment for the 25 ppm treatments. For both drench trials samples were taken at 0, 2 weeks, and monthly for 6 months post-treatment.

Chemical Analysis

Chemical analysis was performed by the CPHST Gulfport Lab Analytical Chemistry Section. The extraction procedures used are based on a 50 gram mixed nursery soil material. An aliquot is transferred and apportioned into hexane. The bifenthrin extract generated is analyzed by Gas Chromatograph (GC) with Electron Capture Detector (ECD) (Figs. 5 & 6). An example of a chromatogram is shown in figure 7. For soil, an additional sub-sample is taken to determine moisture content which is then used as a correction factor to calculate the final concentration of bifenthrin. The limit of detection (LOD) and the limit of quantification (LOQ) for the initial analytical results are 0.9 ppm and 3.0 ppm, respectively.

A set of nursery soil samples is collected by IFA-unit and submitted to the Chain of Custody (COC) unit for processing. The COC unit prepares a packet with sample worksheet, field forms and a technical procedure. A technician or chemist picks up the completed packet and nursery soil sample from COC unit to begin weighing sample for extraction procedure. Controls (composite and 4-LOQ) and each soil sample are then fortified with a known amount of bifenthrin and process standard (methyl chlorpyrifos) to check its % recovery. Samples are extracted by adding 70:30 (v/v) acetonitrile:DI water into the container and capped tightly. The sample is then shaken in a mechanical shaker for 2 hours at low speed. The aliquot extract is then transferred into a separatory funnel, and a 5% NaCL solution and hexane is added and shaken for a minute with venting as needed. The extract is drained through a funnel plugged with glass wool and NaSO4 and repeated 3 times. All extracts are combined and the extract is ready for GC-ECD analysis.

Fig. 5 Gas Chromatograph 6890, monitor (controlled by HP Chem-Station)
Fig. 6 Gas Chromatograph 6890 w/ Electron Capture Detector (ECD)
RESULTS AND DISCUSSION

Incorporated Treatments

All treatment types and rates of application showed a consistent rate of degradation. All started at or near the theoretical dose rate with the exception of the MAFES 25 ppm batch mixed dry treatment which was nearly double the theoretical dose rate initially. This is unexplainable since the subsequent samples appeared to be at or near the same strength as the other 25 ppm treatments. The 10 ppm treatments showed a drop between 0 and 3 months and little change from 3 to 6 months. The 25 ppm treatments showed a similar drop from 0 to 6 months followed by a plateau at 6 and 12 months and another drop at 18 months. Overall, this trial indicates that in the first 12 months after mixing, there is an average decrease in bifenthrin detected by chemical analysis of approximately 41-43%, with a range of 19-72%. Most of this decrease occurs in the first 3 months after mixing (36% decrease). By 18 and 24 months, the decrease in bifenthrin has jumped to 66% of the initial amount with a range of 53-81%. The 10 ppm treatments were terminated after 6 months as scheduled. The 25 ppm treatments were terminated after 24 months. Results are shown in the graphs below:
Drench Treatments

2008

All treatments were somewhat erratic, however, they showed a general decline in concentration over the evaluation period. The chlorpyrifos treatments were considerably more erratic with no discernable trend. This variation is believed to be caused by variations in how quickly each individual pot drained. The slower a pot drains, the longer the insecticide solution remains in contact with the media, which therefore possibly increases the amount of chemical retained by the media. Because each pot was essentially a separate treatment, it is difficult to determine the true rate of degradation that is occurring.
Again the results were somewhat erratic and were more pronounced in the MAFES media. The initial analysis immediately after treatment for both rates of application is significantly lower than the theoretical rates of 10 and 25 ppm with an average of 4.4 and 7.1 ppm, respectively (combining media types and formulations). However, by 2 weeks after treatment, across all media types and formulations, the mean result for the 10 ppm rate is 7.7 ppm and for the 25 ppm rate is 18.8 ppm, 23% and 25% below initial expected theoretical rates. By 3 months after treatment, analyses showed a 54% reduction in initial theoretical dose rate in both the 10 and 25 ppm rates (combined data). At 6 months, the combined data showed a 67-70% reduction in initial theoretical dose rate in both the 10 ppm and 25 ppm rates (combined data).

Conclusions

The application of program chemicals by drenching gives much less consistent chemical concentrations than application by incorporation. Drainage and soil moisture content are factors that are believed to affect the consistency of chemical concentration. The speed at which a pot drains affects the amount of time the chemical solution is in contact with the potting media and therefore affects the amount of chemical that can be bound to the soil. Soil moisture content seems to affect concentration in two ways. The first is by the amount of water occupying binding sites on the media and the second is the effect that moisture content has on drainage.

Because the drench trials provided erratic results, a third trial will be initiated this year. In this trial the media will be drenched in large pots to ensure as consistent application as possible. After treatment the media will subdivided into smaller pots to serve as individual replicates for chemical testing.
INTRODUCTION

The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. Using individual tree drench methods coupled with post-harvest burlap treatment has the advantage of treating trees selectively, with little or no wait time prior to harvest, and avoiding unnecessary treatment to the entire nursery field. Compared with post-harvest dip, this method could avoid the use of heavy equipment and there is no disposal of large volumes of chemical waste.

The objective of this study was to evaluate the combined in-field pre-harvest drench and post-harvest burlap treatment as an alternative IFA quarantine treatment for field grown B&B nursery stock.

MATERIALS AND METHODS

Preliminary trials in MS and in TN

Preliminary trials using tree ring chemogation were conducted in MS and in TN to examine the movement of bifenthrin in two different soil types. Drenches were set up on weedy ground and on active fire ant mounds in both places. Blue dye was used for visually observing how the drench solution penetrates the soil. Colored soil at various depths of the holes and balls after “tree” excavation were collected for female alate bioassay.

Field treatments in MS

Individual tree drench using 5-gal buckets was conducted in a nursery in Lucedale, MS fall 2009. Two buckets were placed at the opposite sides of a tree with drain holes facing toward the tree base (Fig. 1). Buckets were filled half way full and insecticide (bifenthrin 0.025, 0.0125 lb ai or λ-cyhalothrin 0.069, 0.035 lb ai/100 gal) and liquid blue dye were both added to the water; then additional water was added to bring it up to the full 5 gal mark with each tree receiving 10 gals of drench. Eight trees were used in each treatment. Treated trees were machine-harvested the next day with 18” diameter rootball wrapped in plain burlap of 7.5 oz and were brought to Gulfport Lab. Burlap on all rootballs except the control group was sprayed with bifenthrin at 0.05 lb ai/100 gals of water with 2 gal solution for 8 trees in each treatment. Trees were stored outdoors under simulated nursery storage conditions for normal aging.
Bioassay with burlap and soil

A single bioassay cup containing 10 female alates was utilized for each burlap and soil sample (Fig 3. A, B & C). Five replicates were used for each treatment. Female alate mortality was recorded two times a week during the 14-day exposure period, and dead alates were removed during these observations.

Fig. 1. Pre-harvest drenches with 5-gal buckets to boxwood plants in a nursery in Lucedale, Georgia County, Mississippi, fall 2009.

Fig. 2. Tree ring drenches on top of active fire ant mounds with 10 gal solution each in a nursery in Coffee County, Tennessee, spring 2009.

Fig. 3. A & B: individual cups for burlap and soil bioassay. C: a piece of burlap was removed for lab bioassay and soil sample about 1 cm depth was also collected from where burlap was cut out (within yellow square)
RESULTS AND DISCUSSION

Preliminary trials

In our preliminary trials, colors were clearly seen in sandy soil (Fig. 4) but not in clay soil. However, bifenthrin movement in the soil matched that of blue dye in both sandy and clay soils because bioassay results showed that soil samples with 5% or more coloration achieved
100% kill regardless soil type and depth. All active mound drenches in both places were also 100% effective.

*Burlap bioassay*

Consistent results of 100% efficacy at different intervals after the final treatment in our burlap bioassays conducted so far showed that bifenthrin remained in the burlap was potent enough to achieve a quarantine level of control (Fig. 7).

*Soil bioassay*

For all the soil sample bioassays conducted, 100% efficacy was also recorded in all treatments (Fig. 7). Similar results are expected for the 6 month soil bioassay because longer contact with the treated burlap would make the surface soil more effective in killing the newly mated fire ant queens, which was the results of our 2008 intact rootball bioassay.

Despite the difficulty for drench penetration, the tightly packed clay soil could be a positive factor for fire ant control because tunnels within ant mounds in clay soil would allow chemical solution to reach the entire structure, thus killing queens through mound drench since clay soil tends to hold tunnels in better shape (Fig. 6). Both tree rings and 5-gal buckets could be used for chemogation, but advantages of using buckets are: 1. better suited to the "uneven ground" in the field; 2. large mounds cannot be covered by one tree ring but could be by multiple buckets; 3. larger tree trunk does not take tree ring but all sizes of trees can use multiple buckets; 4. no refill is needed for drench of 10 gallons or more using buckets; 5. buckets are more economical than tree rings and are readily available to growers.

**CONCLUSIONS**

1. In-field chemogation with tree rings or 5-gal buckets could penetrate the rootball area in sandy loam soil. In clay soil, however, penetration was less through in the ground but drench solution could reach the entire tunnel system within the fire ant mounds.

2. When applied at 0.05 lb ai per 100 gal water, bifenthrin-treated burlap and surface soil of rootballs could kill fire ant female alates for at least 4 months under normal weathering conditions with longer period of effective control expected.
Evaluation of ARINIX® Permethrin Impregnated Nylon Plastic Strips in Preventing Fire Ant Invasion in RainBird Par + ES Irrigation Boxes at Bear Creek Golf World, Houston, Texas

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Anthony Camerino, County Extension Agent – Horticulture – Harris County
Bastiaan M. Drees, Professor and Extension Entomologist
Alejandro Calixto, Assistant Research Scientist - Southeast District 9
Texas AgriLife Extension Service

Golf is a major industry in the State of Texas. Harris County is home to about 100 golf courses with a total estimated size of 19,800 acres (Camerino, 2007). Golf course maintenance is a high input agronomic system that requires a careful balance between visual aesthetics, turfgrass health, playability and budgetary constraints. Maintaining acceptable conditions is challenging because of a variety of insect pests. A significant insect pest that impacts aesthetics, turfgrass health, and playability is the red imported fire ant, Solenopsis invicta Buren (Hymenoptera: Formicidae). Lard et.al. (1999) determined that golf courses in these five metro areas spent an estimated $30 million on fire ant control, equipment replacement, repair, and medical expenses directly related to fire ant infestation. An infestation can cause a great deal of damage, requiring large sums of money for system replacement or repair. The most costly expenditure, by far, for golf courses was electrical equipment replacement. This amounted to $25.3 million, or approximately 85% of their total annual fire ant related expenditures. Much of this cost ($25.1 million) was for the replacement of irrigation systems that were infested by fire ants.

Bear Creek Golf World (16001 Clay Road, Houston, TX 77084) is a 450 acre daily fee facility located on the west side of Houston, TX that offers 54 holes of golf. Daily fee golf courses, such as Bear Creek Golf World, operate on a very restrictive budget compared to private and semi-private golf facilities. Budgetary constraints and turfgrass health are of more importance to managers of these facilities, while lower standards of aesthetics and playability are tolerated by consumers. This golf facility has a perpetual problem with fire ants infesting its irrigation control box pedestals spread throughout the three 18-hole courses. According to the superintendent, if fire ants could be managed in a way that eliminated damage to susceptible electrical landscape utility devices, such as irrigation controller boxes, the reminder of the impacts caused by fire ants would be acceptable for the expectation level at this golf facility. A study was initiated in 2008 at Bear Creek Golf World to see if a new permethrin impregnated nylon plastic, product named, ARINIX® (www.arinix.com) would prevent fire ants from invading the irrigation control boxes.

NIX of America is a part of a Japanese-based company that has developed ARINIX®. The nylon formulation allows the pesticide to remain embedded in the plastic three to five years depending upon environmental conditions. In laboratory trials, ARINIX® has been shown to help prevent fire ant access to an area when applied as a barrier (Drees, 2007).
Material and Methods

- The ARINIX® product was placed in 20 irrigation controller boxes (Fig. 1 and 6).
- These boxes were located on the Challenger and President courses at Bear Creek Golf World (Fig. 2 and 3).
- Each box was opened, cleaned of dirt and dust, and then numbered for easy identification. On June 18, 2008 the ARINIX product was placed in 15 of the 20 Rain Bird boxes (Fig. 4).
- Three different installation designs of the ARINIX® product were used (Fig. 5).
- Plan 5 (treatment 1) used L-shaped strips but did not utilize ARINIX® spiral wrap around the electrical wires.
- Plan 6 (treatment 2) and plan 7 (treatment 3) not only utilized the ARINIX® L-shaped strips, but 3 inches of the spiral wrap was placed around the electrical wires close to ground level (Fig. 5).
- Plan 7 differed from plan 6 by having double rectangles of L-shaped strips installed in the base of each box.
- Ten observations were taken in a 17 month period.
- Data collected were 1) fire ant workers present in irrigation box (dead or alive), 2) fire ant workers present in irrigation boxes (live only), 3) active fire ant mounds present in irrigation boxes.
- Data from each box were combined over the ten observation dates to determine the effect the presence of the ARINIX® product had on the attractiveness of the irrigation boxes for habitation by the fire ant.
- A Chi-square goodness of fit test was used to analyze the nominal data (frequency distributions, either ants/mounds present or absent and dead or alive) in relation to the treatments (ARINIX® 1, 2, 3) obtained during the trial. In Chi-square, the interest is in the frequency with which individuals fall in the category or combination of categories. All the tests were performed using the statistical package SPSS 16 (SPSS, Inc.). Significant differences when P<0.05 (Tables 1, 2, & 3).

Results and Discussion

- Throughout the duration of the study fire ant pressure around individual irrigation control boxes was moderate to high. Over 70% of the observations taken document that the irrigation control boxes had active fire ant mounds within 15 feet (data not shown).
- The cumulative number of irrigation controller boxes observed to be infested with fire ants (live and dead), live fire ants only, or active fire ant mounds from 10 observation dates over 17 months was monitored (Fig. 7).
- Fire ants were found in irrigation control boxes lined with the ARINIX® strips, but the fire ants present in the ARINIX® treated boxes were dead. No active fire ant colonies were observed in the ARINIX®-treated boxes over the 17 month period (Fig. 8).
- The only live fire ants were found in the untreated irrigation controller boxes. (Chi-square goodness of fit test (P=0.021, α=0.05), Table 1).
- Fire ant workers mortality was significantly higher on ARINIX®-treated boxes (Table 2), and
• Fire ant mounds were significantly less frequent in ARINIX®-treated boxes (Table 3).

Summary

It can be summarized from this study that The ARINIX® product has protected RainBird irrigation controller boxes from red imported fire ant activity for 17 months (Fig. 8). The product does appear to deter colony establishment possibly by preventing foraging fire ants from returning to their respective mounds thus reducing habitation of the treated irrigation box by fire ants. Similar reduction in habitation was seen by Keck et. al. (2007) in San Antonio, TX, where the ARINIX® product was evaluated for the prevention of fire ant invasion in electrical pad-mount transformers.

Conclusions

This product is an effective, reduced-risk, environmentally-friendly, long-term fire ant management option for landscape managers who want to protect specific electrical landscape utility devices, but cannot afford ongoing larger area or whole property fire ant suppression management tactics. In cases where fire ant populations can be tolerated with the exception of damage to a few high value landscape utility devices this management tactic results in far less pesticide applied on a per acre basis compared to the regular spot treatments a contact insecticide would require. In addition, in urban landscapes where property-, area- or community- wide fire ant suppression is not possible due to small property sizes and numerous property owners, this product may be the only feasible option.

Cost estimates for the installation of this product ranges from $30 - $50/ irrigation box, an expense that can be annualized over the life-span of the product’s effectiveness estimated to be 3 to 5 years. Taking the Bear Creek Golf World situation, it would cost from $600 - $1000 in materials to line each of the irrigation control boxes. The usual cost to repair an irrigation box from fire ant habitation can be as little as the manual labor to clean the box, to replacing the electronics of the box at the cost of $500 or greater. At Bear Creek Golf World, just the loss of 1 box/year over the 3-5 year longevity of the treatment would pay for the product.

Literature Citations

Camerino, Anthony. Unpublished survey data. 2007 Annual South Texas Golf Course Superintendents Association meeting, Houston, TX. Texas AgriLife Extension Service – Harris County Office, 2007


Figure 1: Examples of Rain Bird Corporation Par + ES irrigation controller boxes at Bear Creek Golf World located on the Challenger and President courses, Harris Co., TX.
**Figure 2.** Aerial view of Bear Creek Golf World Challenger course with approximate locations of the 9 Rain Bird Par + ES irrigation controller boxes used in 2008 evaluation of ARINIX® product, Harris Co., TX, 2008.

**Figure 3.** Aerial view of Bear Creek Golf World President’s course with approximate locations of the 11 Rain Bird Par + ES irrigation controller boxes used in 2008 evaluation of ARINIX® product, Harris Co., TX, 2008.
Figure 4: Irrigation boxes were cleaned, the ARINIX® product cut to size and then placed in one of three patterns inside the lower portion of the box, Bear Creek Golf World, Harris Co., TX, 2008.

Figure 5. Illustration and randomization of ARINIX® product within Rain Bird Par + ES irrigation controller boxes: “C” represents Challenger course boxes and “P” represents Presidents course boxes; Treatment 1 = Plan 5, Treatment 2 = Plan 6, Treatment 3 = Plan 7, Bear Creek Golf World, Harris Co., TX, 2008.
Figure 6: Various shapes of ARINIX® (www.arinix.com) product used during evaluation in irrigation boxes.

Figure 7: The cumulative number of irrigation boxes observed to be infested with fire ants (live and dead), live fire ants only, or active fire ant mounds from 10 observation dates over 17 months, Bear Creek Golf World, Harris Co., TX, 2008-2009.
Figure 8: RainBird irrigation box lined with ARINIX® product (Plan 6, Treatment 2) compared to irrigation box without ARINIX® product (November 2009), Bear Creek Golf World, Harris Co., TX.

Table 1: Treatment 1 fire ants present - Fire ant workers significantly less frequent on ARINIX®-treated boxes (P=0.021, \( \alpha = 0.05 \), Bear Creek Golf World, Harris Co., TX, 2008-2009.

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**Table 2: Treatment 2 live fire ants present** - Significantly less living fire ant workers in ARINIX®-treated boxes (higher individual mortality) \((P=0.026, \alpha=0.05)\), Bear Creek Golf World, Harris Co., TX, 2008-2009.

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**Table 3: Treatment 3 active fire ant mounds present in irrigation box** - Fire ant mounds significantly less frequent in ARINIX®-treated boxes \((P = 0.026, \alpha=0.05)\), Bear Creek Golf World, Harris Co., TX, 2008-2009.

<table>
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<th>Chi-Square Tests</th>
<th>Value</th>
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Bait Insecticides and Hot Water Drenches against the Little Fire Ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) Infesting Containerized Nursery Plants

Arnold H. Hara¹, Susan K. Cabral¹, Ruth Y. Niino-DuPonte¹, Christopher M. Jacobsen², and Kyle Onuma³

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Introduction

The little fire ant (LFA) *Wasmannia auropunctata* (Roger) was first detected in Hawai‘i in 1999 in the Puna district on the Big Island. Hawai‘i Department of Agriculture attempted to contain the initial infestation; however, the ant had already been inadvertently dispersed by movement of infested potted plants from nurseries. LFA is now widespread between lower Puna and Laupahoehoe, and has been confirmed in the Kona district on the Big Island, as well as single infestations on Kaua‘i and Maui, posing a stinging threat to residents, visitors, and animals (pets, livestock, native birds and mammals), and quarantine concerns for nurseries exporting plant material.

In a field test in the Galapagos on LFA, the preference order for food and commercial ant control baits were: Amdro (hydramethylnon with soybean oil), peanut butter, lard, Raid Max (N-ethyl Perfluorooctane-sulfonamide with peanut butter), Max Force (hydramethylnon on dried silkworm pupae matrix), honey-water, peanut butter oil, and honey; in comparison, Logic (fenoxycarb with soybean oil), and water attracted no *W. auropunctata* workers (Williams and Whelan 1992). Once established, LFA is extremely difficult to control in all but arid, two-dimensional (ground only) simple ecosystems; LFA was eradicated from ca. 21 ha on Marchena Island in a dryland forest area with seasonal 8 months of little or no rain on the Galápagos Archipelago using Amdro (hydramethylnon) fire ant bait (Causton et al. 2005).

The key to preventing recolonization of LFA necessitates control of arboreal as well as terrestrial colonies. Determining the efficacy of several ant baits specifically against LFA must be coupled with research to develop paste or gel bait formulations and means of application along tree trunks and into tree canopies (Souza et al., 2008; Vanderwoude and Nadeau, 2009).

Two trials were conducted to evaluate bait insecticides other than Amdro to control LFA on plant nurseries: 1) broadcasts of hydramethylnon, s-methoprene, and metaflumizone baits, and metaflumizone spray were compared for the control of LFA in potted plants, and 2) the effect of weather-exposure on the efficacy of metaflumizone bait on LFA. In a third trial, LFA susceptibility to hot water immersion was determined, and efficacy of hot water drenching was evaluated as a disinfestation treatment for potted plants prior to transport.
Materials and Methods

Efficacy of several bait insecticides on LFA

This study was conducted at a nursery in Kea’au, HI from February to April 2008 using seedling fishtail palms (15.2 to 30.5 cm tall) growing in 10.2-cm diam. containers. Plants were randomly assigned to one of six treatments: 1) Amdro Pro Bait (0.73% hydramethylnon), 2) BAS 320 I Siesta Fire Ant Bait (0.63% metaflumizone), 3) BAS 320 I Nuisance Sweet Bait (0.63% metaflumizone), 4) Extinguish Plus bait (0.365% hydramethylnon and 0.25% S-methoprene), 5) BAS 320 I 240 SC (24% metaflumizone) applied as a spray, and 6) untreated control. Plants were placed on a table (0.74 m² surface area) standing in soapy water to prevent escape or immigration of new ant colonies and allowed to acclimate for several days prior to taking pre-treatment LFA counts. Treatment reps (12 plants) were isolated on separate tables; each pot was a separate colony. Ants of different colonies foraged together on the table surface but did not mingle within pots. Sprays were applied to the foliage and table surface with a handheld sprayer at 1870.8 liters/ha output; bait granules were evenly dispersed over the table surface. Treatments were applied twice one month apart. All treatments were replicated four times. Foraging LFA were counted in the plants and on the table surface at 2, 7, 10, 14, 21, and 28 d after the first application, and 2, 7, 10, 14, 17 and 21 d after the second application. The plants were then destructively dissected to determine total number of LFA remaining. Based on quarantine standards, a plant was considered infested if at least one live ant was present inside the pot or plant root zone. Data of foraging ants and ants remaining post treatment were log 10 (n+1) transformed; infested plant percentages were arcsine sqrt (p) transformed prior to ANOVA.

Effects of weather-exposure on efficacy of bait insecticides on LFA

A trial was conducted at the University of Hawaii at Hilo, College of Agriculture, Forestry and Natural Resource Management (CAFNRM) instructional farm near Hilo, HI to determine the effects of weather-exposure on the efficacy of Siesta Ant Bait (0.063% metaflumizone) against LFA. Treatments consisted of 1) fresh deposit of Siesta bait; 2) Siesta bait weathered 7 days; 3) Siesta bait weathered 14 days; 4) fresh deposits of Amdro Pro Bait (0.73% hydramethylnon) as a bait standard; and 5) untreated control. There were four replicates per treatment of LFA colonies containing at least 100 workers, one queen, and a small portion of brood (~0.001 g of eggs and pupae). Under field conditions, LFA colonies contain multiple queens; therefore, at least one rep within each treatment had multiple queens. Each replicate colony was transferred to a 20.5 W x 34.5 L x 11.0 H cm plastic container (Iris U.S.A Inc. # 050513); inner walls of the container were coated with 1:1 water: fluoropolymer (Insect-A-Slip) solution, and a thin layer of Tanglefoot Tangle-trap paste (Grand Rapids, MI) was applied along the top edges to prevent ants from escaping. A polystyrene petri dish (60 x 15 mm) (Flacon #35-1007) with blackened lid containing a cotton ball moistened with tap water provided a dark, humid nesting site.

Weather-exposure of Siesta was achieved by placing bait (7 g per rep) on a wire screen (1 x 1 mm mesh) over a 34.3 L x 34.3 W x 7.6 H cm wooden box containing potting media (a layer of potting soil, perlite, and vermiculite mix over a layer of cinder) outdoors on a nursery bench for the prescribed time period. During the 7 d exposure, total rainfall was 6.7 cm with 81% R.H.
and max/min temperatures of 27.8 / 17.8 °C (average temperature 22.8 °C). During the 14 d exposure, total rainfall was 15.5 cm with 77% R.H. and max/min temperatures of 26.7 / 18.3 °C (average temperature 22.5 °C). There was intermittent sunshine alternating with full cloud cover during the 2-wk exposure period.

All LFA colonies were maintained on a diet of sugar water (1:1), peanut butter, and live grasshoppers. Food was withheld for 4 d prior to the introduction of the baits, but water was available throughout the trial. Both weather-exposed and fresh baits were measured volumetrically (20 cc container), at approximately 1/3 of the maximum recommended Amdro label rate for individual red imported fire ant mounds, into aluminum weigh dishes (50 x 13 mm) placed into each colony. The untreated control reps were fed peanut butter, cooked egg, honey, and grasshoppers. The colonies were kept in a room at ambient temperature and relative humidity (average 22.7 °C, 79% R.H.) with natural light.

Observations for mortality were recorded daily for the first three days, then at 3-d intervals over 3 wk. Digital photographs were taken to assist with population counts. Percent mortality of queens and workers relative to initial populations were corrected using Abbott’s formula, arcsine transformed, and then compared using ANOVA and Tukey’s multiple comparison test.

Hot water tolerance of LFA and efficacy of hot water drenching to disinfest potted plants of LFA

An in vitro study was conducted at Waiakea Experimental Station in Hilo, HI to determine the lowest hot water temperature and shortest treatment duration that would be lethal to LFA using a hot water immersion system previously described by Hara et al. (1993). Worker ants were placed into modified polystyrene petri dishes (150 x 20 mm) sealed along the circumference with masking tape (Shurtape Technologies, Inc., Hickory, NC). The cover and bottom of each dish was modified with a 9-cm diam. hole at the center, which were covered with silk organza (74 µm pore size) sealed with hot glue. This modification allowed the exchange of hot water through the petri dish while preventing LFA escape. The dishes were submerged in hot water (45º C) for either 5 min followed by 2 min dip in ambient temperature water (26 °C), or for 10 min followed by 2 min dip in ambient temperature water.. Treated control reps were submerged in ambient temperature water for 12 min (equivalent to the duration of the longest hot water treatment being evaluated). After treatment, the petri dishes were immediately blotted with paper towels and left to dry overnight. Ant mortality counts were taken 24 h after treatment. For the control treatment reps, the petri dish was placed in the center of a large square of paper and surrounded by a barrier of Tanglefoot Tangle-trap paste; the live ants were allowed to climb out of the petri dish but were prevented from escaping by the Tanglefoot barrier. Remaining ants in the petri dish were observed for absence of movement during mortality counts. For the hot water treatment reps, live ants were counted under a dissecting scope. Trial 1 consisted of 4 reps per treatment (300 to 700 worker ants per rep). Trial 2 consisted of 5 reps each of the longer hot water treatment (10 min) and the treated control (300 to 700 worker ants per rep).

To evaluate hot water drenching as a LFA disinfestation treatment of potted plants, naturally-infested fishtail palms Caryota mitis Lour. and rhapis palms Rhapis excelsa (Thunb.) in 15.2 cm diameter plastic pots in media composed of potting soil and volcanic cinder were
randomly assigned (six reps per species per treatment) to one of two treatments: 1) hot water drench at 45.6 °C for 11 min followed by ambient temperature water drench (26 °C) for 5.5 min, or 2) treated control (ambient temperature water drench for 15.5 min). Pre-treatment observations indicated that each plant was heavily infested with LFA (queens, workers, brood). At 2, 5, and 9 days after treatment, a wooden stick lightly smeared with peanut butter was placed in each pot for 30 min, then collected and sealed in plastic bags for counting. At 14 days after treatment, each palm plant was removed from its pot and visually inspected for LFA infestation.

Results and Discussion

Efficacy of several bait insecticides on LFA

There were no differences in pre-treatment counts of foraging worker ants (mean ± SE = 41.1 ± 4.1) or plants infested with LFA (100%). Following the initial treatment applications, foraging behavior was altered within 2 DAT, and treatment differences were detected by 10 DAT (Table 1). While all treatments reduced the initial number of foraging ants after both the first and second applications, applications of BAS 320 I 240 SC spray and Extinguish Plus bait resulted in the greatest and most sustained reduction. Siesta Fire Ant Bait reduced (P<0.01) the number of foraging ants 7 to 10 d after the second application as compared to the untreated check, but its efficacy began to subside by 14 DAT. Efficacy of Siesta Nuisance Sweet Bait was slightly more persistent, abating by 21 d after the first application and by 17 d after the second application. Amdro Pro bait tended to suppress the average number of live LFA throughout the study but levels were lower (P<0.01) than the untreated control only once: at 10 d after the second application.

At the conclusion of the study (7 wk after the first application), only Extinguish Plus and BAS 320 I 240 SC resulted in less (P<0.05) live worker ants and infested plants (%) than the untreated check (Table 2). Nearly all of the plants treated with Siesta Nuisance Sweet Bait were infested with LFA, with mean number of ants as high as the untreated check. While more than 90% of plants treated with Amdro Pro or Siesta Fire Ant Bait were infested with LFA, the average number of ants in the treated plants were reduced by >50% as compared to the untreated check. Also, there was an absence of live queens and brood among plants deemed “non-infested” among all treatments.

From a quarantine treatment perspective, two application of Extinguish Plus bait and BAS 320 I 240 SC applied as a spray, showed potential to be part of a multiple treatment protocol for potted plants infested with LFA, as they completely disinfested 63 to 66% of plants treated, while the infested plants in those treatments averaged only 14 to 29 live worker ants per plant. All of the untreated plants (100%) were infested with >400 live workers and also queens per plant. Complete eradication of LFA with insecticidal baits, granules, and sprays may be accomplished by modifying application frequency, increasing application rates and/or combining these treatments with an effective granular or liquid drench insecticide, such as Talstar (K. Onuma, personal communication).
Effects of weather-exposure on efficacy of bait insecticides on LFA

Initial mortality of worker ants offered fresh or 7 DE Siesta Fire Ant Bait was quick, reaching 91.2% and 71.0%, respectively, by 3 days after treatment (DAT) and rising to 99.7% and 98.6%, respectively, by 20 DAT (Figure 1). Mortality of worker ants offered Amdro Pro or 14 DE Siesta bait rose steadily for the duration of the trial, achieving 84.5% and 90.6% by 20 DAT. For all three Siesta treatments, foraging ceased at 7 DAT. Among the colonies offered Amdro Pro bait, the number of ants observed foraging declined by 6 DAT; at 1 DAT, queens, pupae, and brood were relocated from the nest, which contained bait granules carried by worker ants, possibly indicating potential bait avoidance if the workers were able to act on their preferences under field conditions. The colony was restored back to the nest a day later, possibly due to reduced potency of hydramethylnon from exposure to sunlight.

Mortality rate of queens was higher among colonies offered fresh (77.8%, number of queens (n)=9) or 7 DE Siesta bait (80.0%, n=5) than other treatments (62.5%, n=8 and 44.4%, n=9 for 14 DE Siesta and Amdro Pro, respectively). Replacement queens were observed among the 14 DE Siesta treatment but all died within 20 DAT. Replacement queens observed among the Amdro Pro treatment survived through 20 DAT. There was no queen mortality (n=5) or replacement queens observed in the control treatment for the duration of the trial. Larger trials with more LFA queens are necessary for a conclusive study on effects of bait insecticides on replacement queens.

Efficacy of hot water drenching to disinfest potted plants of LFA

In Trial 1, hot water dipping at 45 °C for 5 min killed only 74% of the ants compared to 99.95% mortality for 10 min, therefore, the shorter treatment duration was not evaluated in Trial 2 (Figure 2). In both trials, hot water dipping for 10 min resulted in nearly 100% mortality. Most of the ants (71.2%) in the treated control were able to survive 12 min. under ambient temperature water, indicating that hot water, and not submersion alone, was efficacious.

After potted palms were drenched with hot water (45.6 °C) for 11 min, no ants were attracted to peanut butter baiting at 2, 5 and 9 DAT as compared to an average of 450 ants per pot in the control group (Figure 3). LFA worker numbers after hot water drenching were reduced by 99.3 and 89.3% in rhapsis and fishtail palm, respectively, as compared to the control drenched with ambient temperature water. When the plants were taken out of their pots for inspection at 14 DAT, the majority of the fishtail palms were found to be severely root-bound which likely affected the extent of contact between hot water and LFA, and thus, lowered mortality rate.

Hot water disinfestation is effective against many vertebrate and invertebrate quarantine pests of nursery plants, and is safe for many heat-tolerant plant species (Hara et al., in press).

Conclusions

Two applications of Extinguish Plus bait (0.365% hydramethylnon and 0.25% S-methoprene) or BAS 320 I 240 SC (24% metaflumizone) as a spray one month apart reduced W. auropunctata foraging worker numbers by >97% as compared to untreated controls. Siesta Fire
Ant bait applied fresh or after 7 days of weather exposure resulted in >98% mortality, and when exposed to weather for 14 d, Siesta was still >90% effective. Efficacy of insecticidal baits and sprays evaluated may be increased by modifying application frequency and/or increasing application rates. Drenching potted plants with 45 °C water for 10 min effectively disinfested them of LFA (>99% mortality), provided the plants were not root-bound to allow adequate contact between hot water and ants. Use of insecticidal baits and sprays along with hot water treatment can be an effective quarantine protocol against little fire ants in containerized nursery plants.

References Cited


Acknowledgements
The authors thank Kris Aoki and Darsen Aoki, University of Hawai‘i CTAHR, for their assistance in conducting the trials.
Evaluation of Potential Imported Fire Ant Quarantine Treatments in Commercial Grass Sod

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Introduction

Imported fire ants (IFA) originated from South America and were accidentally introduced into the United States in the early to mid-1900’s. IFA are now widespread across the Southeastern United States. Movements of this pest are regulated through a system of Federal and State quarantines. Products regulated by the IFA quarantine include but are not limited to hay, nursery plants and other landscape materials including grass sod.

When treating sod in compliance with Federal and State quarantine regulations, sod producer’s options are limited (USDA-APHIS 2006). One option is treatment using the active ingredient (AI) chlorpyrifos at a rate of eight lbs. AI/acre. Currently, no products are registered for IFA control in sod at that required rate. The other option is to use two separate applications of fipronil at 0.0125 lbs. AI/acre applied approximately one week apart. Fipronil is often not an economically viable option for sod producers on a very tight budget. Additionally, the U.S. Environmental Protection Agency has indicated their intention to review and possibly cancel the registration for this material when the conditional registration expires for broadcast granular products containing fipronil used to control imported fire ants. The removal of products containing fipronil, at the rate required for IFA quarantine treatment, will leave no options for sod producers when selling their products to non-quarantined areas and will also prevent the movement of those products across state lines because of Federal quarantine regulations.

Due to limited or costly options available to sod producers, a field study was conducted to evaluate the efficacy of alternative insecticides for use in the IFA quarantine.

Materials and Methods

Evaluations were conducted on a commercial sod farm in Hempstead County, Arkansas. Standard management practices (mowing and irrigation) were maintained throughout the test. No additional herbicides or insecticides were applied to the test area during the conduct of the trial. This trial consisted of 5 treatments arranged in a Randomized Complete Block Design (RCBD) with 3 replications. Treatments tested are listed in Table 1.
Prior to treatment initiation, 15 half acre plots were marked by flagging plot corners. Each plot center was marked by staking a small plastic tag flat to the ground so as not to impede regular mowing and the GPS coordinates for each plot center marker were recorded to aid in relocating plot centers throughout the test. Plot center markers were used as an anchor point for a 58.9’ cord that delineated the radius of a ¼ acre circle in the middle of each plot. All ratings were made within this ¼ acre circle. Pretreatment counts were made on 06/04/09. Treatments were then initiated with application sequence #1 on 06/04/09, followed one week later with application sequence #2 on 06/11/09, followed one week later with application sequence #3 on 06/18/09. All applications were made with a towed sprayer applying a finished spray at 20 GPA (15 ft. boom with ten 8003FF nozzles on an 18” spacing at 20 psi and 5.2 MPH).

Post-treatment efficacy was evaluated by determining red imported fire ant (RIFA) activity in mounds within the ¼ acre center circle of each plot. Each mound was gently probed with a small diameter wire (minimal disturbance technique) and the number of ants responding within 20 seconds was estimated (Jones et al. 1998). Mounds were considered inactive if there were less than 25 RIFAs responding to the probe (USDA Mound Activity Rating Scale).

Post-treatment ratings (No. Active Mounds/Acre) were made on 06/22/09 4 DALA (Days After Last Applic.), 06/25/09 7 DALA, 07/02/09 14 DALA, 07/09/09 21 DALA, 07/16/09 28 DALA, 07/23/09 35 DALA, 07/30/09 42 DALA, 08/06/09 49 DALA, 08/13/09 56 DALA, 08/26/09 69 DALA, and 09/10/09 84 DALA (or 12 weeks after the last application).

All data were analyzed using Gylling’s Agriculture Research Manager Software (ARM 7.0.3. 2003). Analysis of variance was run and Least Significant Difference (p=0.05) was used to separate means only when AOV Treatment P(F) was significant at the 5% level.

**Results and Discussion**

The efficacy of potential quarantine treatments in reducing the number of active RIFA mounds per acre is given in Table 2 and Figure 1. There were no statistically significant differences among treatments with regard to RIFA mound density when rated (pretreatment) prior to the application of any control materials. Average pretreatment density of red imported fire ant mounds in the test area ranged from 25.2-33.2 mounds/acre.

At seven DALA, all treatments had significantly fewer active mounds than did the untreated control and the plots treated with three applications of Hero™ and those treated with Advion™/Onyx Pro™ contained zero active mounds per acre. At 28 DALA there were no longer any significant differences between the untreated control and any of the treated plots, and those plots which previously had zero active mounds had active mounds at 28 DALA or the week before at 21 DALA. The lack of significant differences may have been due to hot, dry weather which lowered fire ant activity in all plots thus possibly masking treatment differences that previously existed. Two applications of Hero™ reduced the number of active mounds to zero by 21 DALA, but active mounds were present the next week.
All insecticide treatments significantly reduced the number IFA colonies in treated plots and did so to a level acceptable for most commercial IFA management situations. However, IFA quarantine treatments for use in commercial sod production must reduce the IFA population to zero within a reasonable period of time and maintain 100% control for at least six weeks to be considered an acceptable quarantine insecticide treatment. The combination of a bait and residual insecticide treatment into IFA/sod quarantine treatment options appears promising. Future studies evaluating differing treatment regimes incorporating IFA insecticidal baits with residual contact insecticides are warranted.

References Cited


Table 1. Insecticides tested in the IFA quarantine treatment evaluation study. Hempstead Co. AR. 2009.

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Table 2. Efficacy of potential quarantine treatments in reducing the number of active RIFA mounds per acre. Hempstead Co., AR. 2009.

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Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Figure 1. Efficacy of potential quarantine treatments in reducing the number of active RIFA mounds per acre. Hempstead Co., AR. 2009.
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<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>3:00PM - 5:30PM</td>
<td>Registration (2nd Floor Lobby)</td>
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<tr>
<td>6:30PM - 8:30PM</td>
<td>Evening Reception (Salon A &amp; B)</td>
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<tr>
<td>3:00PM - 8:30PM</td>
<td>Poster Setup (Palisades)</td>
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</tbody>
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### TUESDAY - April 20, 2010 (General Session – Salon D)

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:00AM - 8:15AM</td>
<td>Breakfast (provided in Salon A &amp; B)</td>
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<tr>
<td>7:30AM - 5:00PM</td>
<td>Registration (2nd Floor Lobby)</td>
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<tr>
<td>8:15AM - 8:30AM</td>
<td>Welcome by Dr. Rob Wiedenmann, Introductions, Local Attractions</td>
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<tr>
<td>8:30AM - 10:00AM</td>
<td>RASBERRY CRAZY ANT SESSION</td>
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<tr>
<td>8:30AM - 8:45AM</td>
<td>1) Rasberry Crazy Ant Spread, Control and Impact. Tom Rasberry</td>
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<tr>
<td>8:45AM - 9:00AM</td>
<td>2) Applied Research Update: Rasberry Crazy Ant. Bastiaan M. Drees, Danny McDonald, Paul Nester, Ajeandro Calixto, Corrie Bowen and Roger Gold</td>
</tr>
<tr>
<td>9:30AM - 10:00AM</td>
<td>5) Rasberry Crazy Ant Session: Open Discussion. Lead by Bastiaan M. Drees, Tom Rasberry, Dawn Calibeo-Hayes and Linda Hooper-Bui</td>
</tr>
<tr>
<td>10:00AM - 10:30AM</td>
<td>Morning Break</td>
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<tr>
<td>10:30AM - 10:45AM</td>
<td>REGULATORY</td>
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<tr>
<td>10:30AM - 10:45AM</td>
<td>6) Overview of USDA Imported Fire Ant Program and Regulatory Issues in the U.S. Stacy Scott</td>
</tr>
<tr>
<td>10:45AM - 11:00AM</td>
<td>EXTENSION/EDUCATION</td>
</tr>
<tr>
<td>10:45AM - 11:00AM</td>
<td>7) Fire Ant Mythbusting with Social Media. Margaret Lawrence and Kathy Flanders</td>
</tr>
</tbody>
</table>
11:00AM - 12:00PM  BIOLOGICAL CONTROL

11:00AM - 11:15AM  8) Preparations for the Field Release of the Little Decapitating Fly, *Pseudacteon cuttellatus* in Florida. **Sanford D. Porter**, Luis A. Calcaterra, Laura Varone and Juan Briano

11:15AM – 11:30AM  9) Spread of Fire Ant Decapitating Flies *Pseudacteon tricuspis* and *Pseudacteon curvatus* in Louisiana. **Anna Meszaros** and Seth J. Johnson

11:30AM - 11:45AM  10) Fire Ant Baits and Biocontrol with Pathogens Update. **David Oi**

11:45AM - 12:00PM  11) Effects of Fire Ants on an Important Biological Control System. **S.B. Vinson** and J. Chantos

12:00PM - 1:00PM  Lunch (provided in Salon A & B)

1:00PM - 2:30PM  BIOLOGY/ECOLOGY

1:00PM - 1:15PM  12) Comparing Bait Mediated Behavior Justifications in Disturbed and Undisturbed Fire Ant Colonies. **Xing Ping Hu**

1:15PM - 1:30PM  13) Characterization and Impact of Picorna-like Virus from the Red Imported Fire Ant (*Solenopsis invicta*) in Taiwan. **Hung-Wei Hsu** and Chen-Jen Shih

1:30PM - 1:45PM  14) Application of Red Imported Fire Ants (*Solenopsis invicta*) Virus (SINV1) Phylogeny to Ascertain the Origin Site of Infection in Taiwan. **Wen-Cheng Yang** and Cheng-Jen Shih

1:45PM - 2:00PM  15) Red Imported Fire Ants Discard Cricket Eggs. **Jian Chen**

2:00PM - 2:15PM  16) Aggression Behavior of Red, Black and Hybrid *Solenopsis* Fire Ants May Explain Their Shifting Spatial Distribution in the Southern United States. **Henry Fadamiro**, Xiaofang He and Li Chen, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

2:15PM - 2:30PM  17) Fire Ant Predation and the Effects of Fire Ant Management on White Grubs (Coleoptera: Scarabaeidae) in Managed Turfgrass. **David Held** and S. Addison Barden

2:30PM - 3:00PM  Afternoon Break

3:00PM - 3:45PM  BIOLOGY/ECOLOGY (continued)


3:15PM - 3:30PM  19) Ecology and Economics of Post-Hurricane Fire Ant
Perturbations in Louisiana Sugarcane. Julien M. Beuzelin, A. Meszaros and T. E. Reagan

3:30PM - 3:45PM 20) Co-existence of Red and Hybrid Fire Ants in Middle Tennessee. S. Ochieng, F. Mrema and J. Tyus

3:45PM - 4:15PM SPONSOR/INDUSTRY PRODUCT DISCUSSION

4:15PM - 5:00PM VIEW POSTERS WITH PRESENTERS (Palisades)

5:00PM Adjourn

Evening Dinner (on your own)

WEDNESDAY - April 21, 2010 (General Session – Salon D)

7:00AM - 8:00AM Breakfast (provided in Plaza Bar & Grille)

8:00AM - 9:00AM KEYNOTE ADDRESS
Behavior and Ecology of Ants in the Tropical Forest Canopy. Stephen P. Yanoviak

Dr. Steve Yanoviak is Assistant Professor, Department of Biology, University of Arkansas at Little Rock. Dr. Yanoviak received his Ph.D. in zoology in 1999 from the University of Oklahoma, his M.S. in entomology in 1993 from Purdue University and his B.S. in entomology in 1991 from Auburn University. His research interests include how species interactions, community processes and anthropogenic disturbances shape local species richness in terrestrial and small aquatic systems. Dr. Yanoviak’s approach is field-based and focuses on the behavioral ecology of arthropods and trophic ecology of detritus-based systems. His current projects address: 1) behavioral ecology of tropical arboreal ants and, more generally, selection pressures associated with life in arboreal settings; 2) community ecology of leaf litter and phytotelm systems; and 3) ecology of disease vectors, especially mosquitoes. Details on his research projects are at: http://www.canopyants.com. Contact Information: Department of Biology, UALR, 2801 S. University Ave. Little Rock, AR 72203-1099, Phone: (501) 569-8342, Fax: (501) 569-3271, Email: spyanoviak@ualr.edu

9:00AM - 10:00AM MANAGEMENT

9:30AM - 9:45AM  23) Broadcast and Skip Swath Bait Treatment Effects on Fire Ant Mound Height and Density. Charles L. Barr and Sam Womble

9:45AM - 10:00AM  24) Baits and Sticks: A Successful Fire Ant Management Combination. Chuck Browne, L. C. "Fudd" Graham, Kathy Flanders, and Kelly Ridley

10:00AM - 10:30AM  Morning Break

10:30AM - 11:30PM  PANEL DISCUSSION

10:30AM - 11:30PM  25) Gaps in Knowledge in Research and Extension in Fire Ants. Linda Hooper-Bui, Kathy Flanders, Jian Chen and Xing Ping Hu

11:30PM - 12:00PM  BUSINESS MEETING (Salon D)

12:00PM - 1:00PM  Lunch (provided in Salon A & B)

1:00PM - 1:30PM  VIEW POSTERS WITH PRESENTERS (Palisades)

THURSDAY - April 22, 2010 (Palisades)

8:00AM -12:00PM  eXtension Work Day

POSTER PRESENTATIONS (Palisades)

DSP01  Distribution of Pseudacteon curvatus and Pseudacteon tricuspis in Arkansas. Jake M. Farnum and Kelly M. Loftin


DSP03  RIFA Effects on Some Native Ants in Arkansas Prairies. Lynne C. Thompson and David M. General

DSP04  Multigenes Expressed in Response to Bait Stress in Solenopsis invicta. Liming Zhao, Jian Chen, Xixuan Jin, James Becnel, Gary Clark and Kenneth Linthicum

DSP05  Sensilla on the Tarsi and Ovipositor of the Phorid Fly, Pseudacteon tricuspis May Play a Role in the Location and Attack of Fire Ant Workers. Kavita Sharma and Henry Fadamiro

DSP06  A Characterization of m+DNA Haplotypes for Two Imported Fire Ant Species and Their Hybrid. David C. Cross and Michael A. Caprio

DSP07  Worker Ant Foraging Response on and Near Mounds of the Red Imported Fire Ant. Kimberly Schofield, Bastian M. Drees, and Bill
Summerlin

DSP08 Arginine kinase: Differentiation of Gene Expression and Protein Activity in the Red Imported Fire Ant, *Solenopsis invicta*. **Nannan Liu**, Haichuan Wang, Lan Zhang, Lee Zhang, and Qin Lin

DSP09 Using Fire Ant Demonstrations to Open the School IPM Door. **Karen M. Vail**, J. G. Chandler, P. A. Barnwell and J. C. Maples

DSP10 Degradation of IFA Program Chemicals in Various Potting Media. **Lee McAnally** and Anne-Marie Callcott


DSP12 Evaluation of ARINIX® Permethrin Impregnated Nylon Plastic Strips in Preventing Fire Ant Invasion in RainBird Par + ES Irrigation Boxed at Bear Creek Golf World, Houston, Texas. **Paul R. Nester**, Anthony Camerino, Bart Drees, and Alejandro Calixto


DSP14 Controlling Fire Ants in Pastures Demonstration. **Richard Lee Van Vlake**, Russell Duncan, and Tim Davis


DSP16 Recent Studies on Fire Ant Chemical Ecology. **Robert Vander Meer** and Man-Yeon Choi
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