Area-Wide Suppression of Invasive Fire Ant *Solenopsis* spp. Populations

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ABSTRACT The fire ants *Solenopsis invicta* Buren and *Solenopsis richteri* Forel were inadvertently introduced into the USA early in the 1900s and currently inhabit over 150 million hectares in Puerto Rico and twelve southern states from Texas to Virginia. Imported fire ants have also become established in isolated sites in Arizona, California, Maryland, and New Mexico. The large numbers and potent sting of fire ants have resulted in significant medical, agricultural, and environmental impacts. The population densities in the USA are five to ten times higher than in South America, most likely due to their escape from natural enemies. Recently, biological control agents have become available in the USA, e.g. *Pseudacteon* spp. decapitating fly parasitoids and a microsporidian pathogen *Thelohania solenopsae* Knell, Allen & Hazard, setting the stage for integrated fire ant management. An area-wide fire ant management project proposal was funded by United States Department of Agriculture-Agricultural Research Services headquarters to demonstrate control of fire ant populations over large areas using commercially available insecticide baits and self-sustaining biological control agents. Untreated, bait control and integrated pest management (IPM) demonstration sites (120 hectares and periphery) were set up in each of five states (Florida, Mississippi, Oklahoma, South Carolina and Texas). The control and IPM sites both had bait (hydramethylnon and methoprene) applications, but only the IPM site had biological control agents released around the periphery. After 3.5 years, decapitating fly parasites have been established at the demonstration sites. The microsporidian pathogen is established in all sites except Mississippi. Fire ant populations have been reduced by 85-99% in the IPM demonstration sites as compared to untreated areas of the same sites. Environmental assessment has demonstrated that bait toxicants do affect non-target ant species but do not affect the arthropod species richness. Educational outreach activities resulted in informative brochures, the establishment of a programme web site, videos, and general information on fire ants. The research component has responded with additional biological control organisms and better pathogen detection methods.

KEY WORDS fire ants, *Solenopsis invicta*, *Solenopsis richteri*, baits, *Pseudacteon* spp., *Thelohania solenopsae*, area-wide, integrated pest management

1. Introduction

The fire ants *Solenopsis invicta* Buren and *Solenopsis richteri* Forel were inadvertently introduced into the USA in the early 1900s and currently inhabit over 150 million hectares in Puerto Rico and twelve southern states from Texas to Virginia. Imported fire ants have also become established in isolated sites in Arizona, California, Maryland, and New Mexico (Fig. 1) (Callcott and Collins 1996, CFR 2001). They have recently widened their invasive character through accidental importation and establishment in Australia, China, Hong Kong, and Taiwan.

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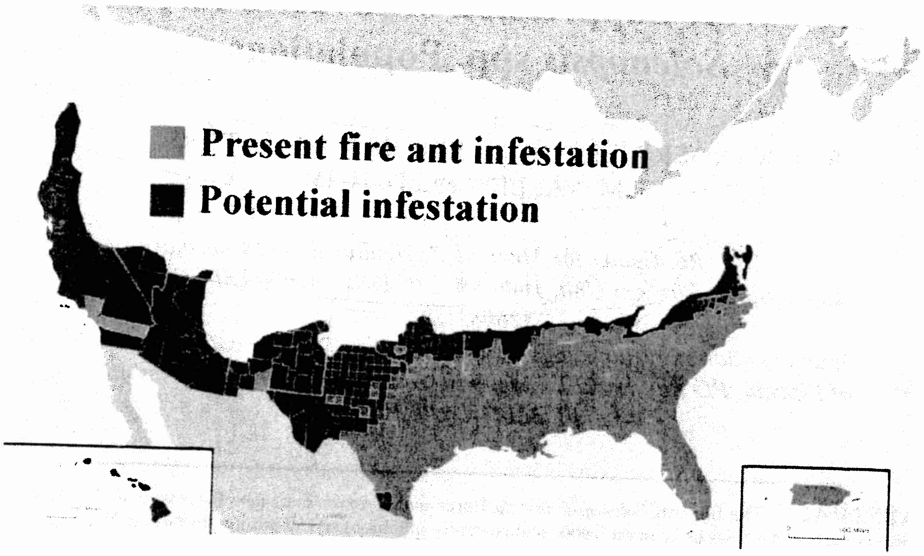

Figure 1. *Present fire ant infestation in the USA and potential infestation based on an ecological model using temperature as limiting factor for fire ant development.*

1.1. Economic Impact

Fire ant colonies (single queen), can contain up to 250 000 workers and reach infestation rates of over 130 mounds per hectare (Tschinkel 1988). More recently, multiple-queen colonies have proliferated in the southern states with even greater nest and population densities (Macom and Porter 1996). The fire ants' large numbers, resource requirements, aggressive behaviour, and potent sting have resulted in many negative interactions with humans and the ecosystem. The fire ant is an opportunistic omnivore that affects agriculture in many ways. The ant damages germinating seeds and root systems of row crops (Shatters and Vander Meer 2000), negatively affects citrus (Adams 1986), and causes USD 67 million in annual losses to cattle producers in Texas alone (Barr and Drees 1996). More than 30% of the human population in infested areas is stung each year and because venom hypersensitivity occurs in 1% of the population, hundreds of thousands of people may require medical attention each year (deShazo et al. 1999). To date around 80 people have died in the USA due to fire ant stings. This highly aggressive ant reduces populations of native ants, other insects, and destroys many small animals including endangered species (Porter and Savignano 1990). The economic impact of fire ants to the US economy is estimated at more than USD 5500 million annually in damage, medical treatments, and control (Fig. 2) (Pereira et al. 2002).

1.2. Chemical Control

Several mound drenches have been developed for fire ant control, but are impractical on a large scale. The most effective and environmentally safe method of control is the use of toxic baits. Toxic bait development by the chemical industry has primarily focused on the lucrative urban/homeowner market, thus few companies have pursued registration of baits for use in agricultural settings. Even when available, toxic baits are expensive and their non-specific nature adversely impacts non-target native ant species, as well as the environment. Chemical treatment strategies alone are not a viable option for large tracts of land (pastures).

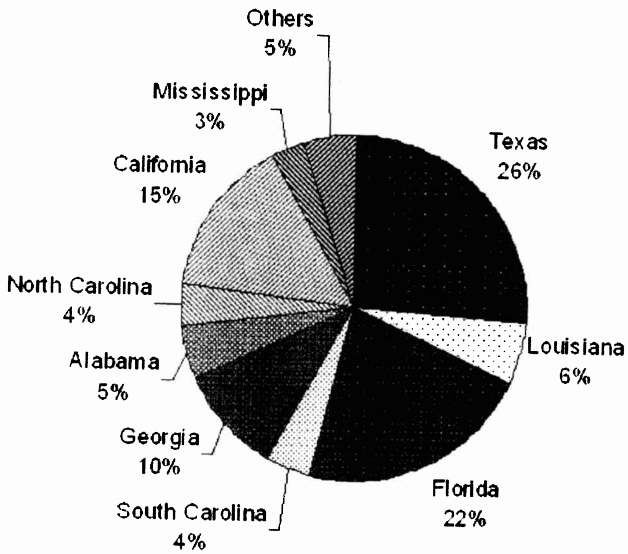

Figure 2. *Pie chart showing the percentage of annual fire ant economic impact on the US economy by state. Total impact is estimated at more than USD 5500 million per year for cost of treatment, damage to property, medical treatments, and all other costs.*

1.3. Biological Control

Fire ant densities in the USA are five to ten times those normally found in their native South American homeland. Thus, it appears that fire ants in the USA have escaped the effects of numerous natural enemies that were left behind in South America (Porter et al. 1992, Porter et al. 1997). Over the past two decades the United States Department of Agriculture (USDA) has actively pursued the identification, importation, and release of selected biological control agents (Williams et al. 2003), such as microsporidian pathogens and decapitating *Pseudacteon* spp. flies (Porter et al. 2004, Vazquez et al. 2006). These biological control agents are important stressors to fire ant populations and can impact them over wide areas. *Pseudacteon* spp. flies are very host-specific (Porter and Gilbert 2004), completing their development within the fire ant heads and causing them to fall off. The flies cause direct mortality, but most important may be the reduction of foraging and consequent weakening of fire ant colonies (Mehdiabadi and Gilbert 2002). Microsporidian pathogens *Thelohania solenopsae* Knell, Allen & Hazard and *Varimorpha invictae* Jouvenaz & Ellis are also very host-specific. They cause chronic diseases in workers and reproductives. *T. solenopsae* causes slow colony death (Williams et al. 1999), and infected ants are more susceptible to the insecticide hydramethylnon (Valles and Pereira 2003).

2. Area-Wide Suppression of Fire Ants

With the availability of self-sustaining biological control agents and effective toxic baits, the Fire Ant Unit applied for and received competitive funding for development of an integrated pest management (IPM) system for area-wide suppression of fire ants through a USDA-

Agricultural Research Service (ARS) headquarters-funded programme entitled "Crop Protection and Quarantine". The objective of this programme is to promote the integration of biological, genetic, cultural, physical, and chemical control technologies into effective, economical, and sustainable IPM systems and area-wide suppression programmes so that they can be transferred to customers as effective management programmes to insect and mite problems.

2.1. Goal and Potential Benefits

The overall goal of the project is to maintain low fire ant populations with reduced need for bait toxicants by using available self-sustaining fire ant biological control agents in conjunction with bait toxicants (Pereira 2004). Anticipated benefits include: (1) demonstration of practical and long-term area-wide control of fire ant populations, (2) quantification of regional differences in treatment frequency to maintain fire ant control, (3) development of decision-making tools for efficient timing of bait toxicant treatments, (4) reduced pesticide risk, (5) sustained fire ant population reduction, (6) increased farm worker safety, (7) lower livestock production costs, (8) establishment of ongoing partnerships with land managers that foster even broader area-wide fire ant management in the future, (9) transfer of area-wide management technology to state agencies and to federal, state, and private land managers, (10) providing a model area-wide fire ant management programme that can be utilized beyond pastures and the cattle industry, (11) restoring the ecological balance toward the native fauna, and (12) providing a better understanding of the economics of fire ants in the cattle industry. To determine if any of these potential benefits are being realized requires measurement of fire ant populations, monitoring the environmental impact of fire ants and the effects of their control on native ant fauna, developing educational materials on fire ant control options, and assessing the economic impact associated with fire ants and the benefits of fire ant control.

2.2. Programme Management Plan

The area-wide project for fire ant population suppression in pastures is coordinated by a management team composed of scientists from the USDA-ARS, the USDA-Animal Plant Health Inspection Service (APHIS) (Gulfport Plant Protection Station, Gulfport, MS), land grant universities, and state agencies. USDA-ARS scientists provide the central leadership in directing major activities associated with executing the project in the five diverse locations chosen for this demonstration. State members of the management team are charged with the development of the within-state infrastructure needed to carry out the complex pre- and post-treatment assessments required for evaluation of project success.

2.3. Demonstration Site Methodology

Demonstration sites were established in pastures in Florida, Mississippi, Oklahoma, South Carolina, and Texas. All sites were established in improved pastures with multiple queen fire ant populations (*S. invicta*), except in Mississippi where single queen black (*S. richteri*) and hybrid (Vander Meer et al. 1985) fire ant populations were present. Each demonstration site consisted of a central area of between 60-120 hectares where fire ant populations were controlled with the bait combination mentioned below, and a surrounding area that received no bait treatment. The surrounding areas served as negative controls and as the sites for establishment of biological control agents. Thus, two types of sites were established in each state: (1) the IPM site where biological control agents were established and the bait combination was used (Fig. 3), and (2) the control site where only the bait combination was used.

At each of the sites, sampling plots were marked both in the bait-treated area and in the surrounding untreated area. These plots consisted of 500 square metre circular areas, with the centre permanently marked on the soil and geo-referenced using a global positioning sys-

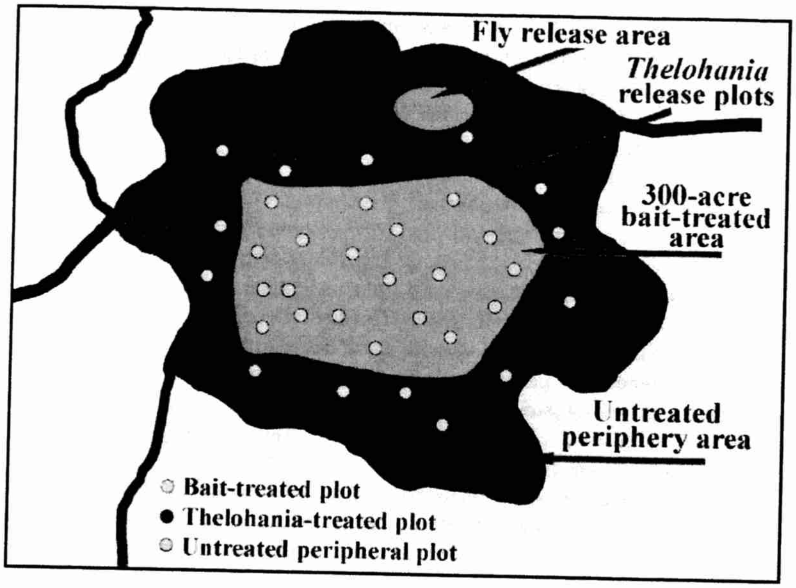

Figure 3. *Schematic drawing (not to scale) of IPM demonstration site with its different components. All bait applications were done by aircraft.*

tem. Several measurements were taken in these plots in order to monitor the fire ant population, the presence and spread of the fire ant pathogen *T. solenopsae*, the decapitating flies, and the non-target arthropod population.

2.3.1. Population Assessment

Fire ant population monitoring was done using two techniques: (1) mound counts and rating, and (2) ant activity monitoring lures. Active fire ant mounds found in the sampling plots were scored using a population index rating system (Lofgren and Williams 1982). The population index rating varied from one to ten. A pre-established bait treatment threshold of 50 mounds per hectare with a population index higher than seven was used to trigger application of the bait combination. Ant activity was monitored by deploying ten pieces of processed meat sausage in each of the sampling plots. After 30-60 minutes, these food lures were observed for the presence of fire ants or other ants. The fire ant population was estimated using the number of ants per lure and the percentage of lures with fire ants.

2.3.2. Bait Treatments

A combination of baits containing hydramethylnon and methoprene (1:1) was chosen for use in the demonstration sites to take advantage of two different modes of action. This combination has been tested extensively with excellent results in Texas (Barr 2002). Hydramethylnon offers a much quicker effect on the fire ant population with mortality occurring within a week or so but without much residual effect. Methoprene causes a decrease in reproductive potential without immediate effect on the fire ant population. The combination of the two active ingredients provides for a quick but lasting effect on the fire ant population. Importantly, these products are registered for grazed cattle pastures and could be later adopted by farmers. They also have little impact on the non-target fauna in the demonstration sites, which is important since the rebound of the natural population of arthropods, including competing native ant populations, is an expected result of the fire ant management programme.

All bait applications were done using aircraft (Fig. 3). After the initial treatment no further applications were done until the population threshold of 50 mounds per hectare was reached or surpassed. Baits were applied at a rate of 1.7 kilogram of 1:1 hydramethylnon and methoprene mixture per hectare. Four weeks after bait application, the fire ant populations were assessed to determine the effectiveness of the bait application.

2.3.3. Biological Control Establishment

The decapitating flies were released at the IPM sites either as adult flies (*Pseudacteon tricuspis* Borgmeier) distributed over partially excavated mounds or in laboratory-parasitized ants returned to their mother colonies (*Pseudacteon curvatus* Borgmeier, *Pseudacteon litoralis* Borgmeier). The total numbers of parasitized ants released at each site were approximately 4000-6000. Decapitating flies were considered established at a location when active flies were observed hovering over disturbed fire ant mounds in the spring or summer after surviving a winter in the field. Initially, fly presence was monitored just in the release areas, but after clear indication of establishment, fly presence was monitored at increasing distances from the release sites.

T. solenopsae inoculations were made within active fire ant mounds by introducing approximately three grams of live, *T. solenopsae*-infected brood per mound in areas where the microsporidian was not yet established at the start of the demonstration project (Mississippi and South Carolina). Worker ant samples were subsequently returned to the laboratory, macerated, and examined for *T. solenopsae* spores using a phase contrast microscope (Williams et al. 1999). *T. solenopsae* was considered established when spores were found in samples more than six months after inoculation.

2.3.4. Environmental Assessment

The environmental effect of the fire ant control programme was assessed by monitoring the surface-active arthropod population using pitfall traps. In the spring and fall of each year, four traps were deployed in each sampling plot for 48-72 hours, and then processed in the laboratory. Of special interest was the presence and number of native ants that could impact fire ant reinfestation rates. Insects, arthropods and other small animals that fell into the pitfall traps were tabulated by morphospecies.

3. Results

3.1. Research Component

The research component of the area-wide project is not critical to the project's success, but if successfully implemented the results would facilitate the attainment of project goals. One example is given on improved pathogen detection.

A major component of the red imported fire ant (*S. invicta*) area-wide project was augmentation with the microsporidian pathogen, *T. solenopsae*. Evaluations to detect successful spread of this pathogen were limited to microscopic examination of macerated ants for the presence of the characteristic spore stage. Unfortunately, this method is labour intensive and cannot detect incipient infections because of the absence of spores. In order to improve detection of all stages of infection of *T. solenopsae* in fire ants, a multiplex polymerase chain reaction (PCR) method was developed. Oligonucleotide primers were designed to unique areas of the 16S rDNA gene of *T. solenopsae* and a region of the *Gp-9* gene of *S. invicta*. Multiplex PCR resulted in sensitive and specific detection of *T. solenopsae* infection of *S. invicta*. The *T. solenopsae*-specific primer pair only amplified DNA from *T. solenopsae* and did not result in amplification products from DNA preparations from uninfected *S. invicta*. The *Gp-9* specific primers recognized and amplified DNA from monogyne and polygyne *S. invicta* social forms, but not from *T. solenopsae*, and, as such, served as a positive control verifying successful DNA preparation. Multiplex PCR detected *T. solenopsae* in all *S. invicta* life stages. *T. solenopsae* could be detected in workers with only ten spores if the *T. solenopsae*-specific primer was used. Multiplex PCR detection of *T. solenopsae* offers the advantages of a positive control, a single PCR amplification, detection of all developmental stages of *T. solenopsae*, and increased sensitivity and specificity compared with microscopy (Valles et al. 2003).

3.2. Educational Activities

The objective of the educational component is to educate the public on fire ant biology, impact and control, including chemical and biological control agents. A web site (http://www.ars.usda.gov/fireant/) for the project was created and updated continuously with new information. Educational videos describing the fire ant disease caused by *T. solenopsae* and the decapitating flies were produced and distributed both via the web site and in a compact disc playable on any computer or other players attached to a television monitor. Also brochures explaining the project concept and objectives, as well as the biological control agents were produced and distributed by direct mailing, insertion in trade magazines, and direct distribution to the public on several occasions, including state agricultural fairs. All these materials have been used in conjunction with slide shows and other public presentations on the project and its various components.

Public interest in all these materials has been enormous, and their response to the information on the biological control agents, especially the decapitating flies, has been very good. Part of the video describing the parasitic decapitating fly was the subject of a very positive article by a nationally syndicated columnist. This article caused a huge influx of requests to the project web site, requiring it to be moved to a more robust server. At agricultural fairs where the decapitating flies were displayed, the public showed great curiosity and support for the research and use of these flies to control fire ants. It is anticipated that the web site will continue to be maintained after the project formally ends, in order to provide continuity to current and future users of the technology. The educational component of the area-wide project has been very successful as indicated by the high level of public knowledge of fire ants, the individual projects, and by high use of the web site.

3.3. Environmental Impact

Pitfall traps were used to evaluate the environmental impacts of treatments at the demonstration sites on the abundance of non-target ants and the species richness of other surface-active arthropods. For the Florida sites the results were mixed concerning non-target ant abundance. The IPM site usually had more native ants in the plots treated with fire ant baits than in the untreated surrounding plots, while at the control site it was generally the reverse, but this was not consistent over time. The number of non-target ant species in the areas treated by baits was about 20% lower at both Florida IPM and control sites, but again this trend was not consistent over time. The species richness of non-ant arthropods collected in the pitfalls did not differ between plots treated with baits and surrounding untreated plots.

Preliminary evaluation based on these data indicates that the bait treatments did not affect the overall richness of arthropod morphospecies, although there is evidence, though not always consistent, for a modest reduction in the richness and abundance of non-target ants. In the 2005-2006 season, the data for all five sites will be assembled in order to compare sites with and without fire ant biological control agents.

3.4. Economic Assessment

Economic surveys were prepared by an agricultural economic team from the Texas A&M University and sent to the farmers involved in the demonstration sites as well as the researchers in each state. These surveys assessed the impact of the fire ant pests on farm activities, as well as the costs and benefits of the area-wide IPM project. These surveys are being analysed, and the data obtained so far have been used to estimate the economic impact of fire ants on the US agriculture and on the economy of individual states. Estimated impacts in Texas and Florida represent approximately 50% of the impact of fire ants in the

USA, with the rest divided among the other infected states. The estimated value for California assumes the infestation is not eradicated.

4. Measures of Success

Evaluation of the following expected outcomes three years after project implementation can serve as a measure of project success:

4.1. Release and Spread of Biological Control Agents

Decapitating flies have been released and established in all states where the area-wide project has been implemented, and in several other locations throughout the USA. Three decapitating fly species have been released and others will be soon.

4.2. Sustained Fire Ant Control

Fire ant populations at the demonstration sites have been maintained at less than 20% of pre-treatment levels throughout the project duration, and farmers are aware of a noticeable decrease in the fire ant population. Results in Florida and Texas indicate that the IPM approach provides advantages over the pesticide-only approach. In Florida, fire ant control averaged 88% where the IPM approach was used as compared to only 71% where fire ants were controlled only by chemical pesticides. In Texas, plots with high decapitating fly populations had decreased fire ant populations as compared to plots with low or no decapitating fly presence.

A simplified method for evaluation of fire ant populations has been developed using food lures. This method makes fire ant control over an area easier, less costly, and more efficient.

4.3. Lower Livestock Production Costs

Area-wide project economists have not yet been able to estimate benefit/cost ratios for the project, mainly due to the short duration of the project.

4.4. Restored Ecological Balance among Native Ants, Birds, and Wildlife

No effects have been observed on the abundance of other arthropods. However, this may change after a longer period with low fire ant populations.

The current demonstration sites will continue to be monitored, but under a less intensive regime. This is possible because of the information gathered from the intense monitoring of the sites during the first three years of the project.

4.5. Increased Farm Worker Safety and Reduced Pesticide Risk

Decreased fire ant populations and the use of low-risk bait toxicants increases farm worker safety and decreases pesticide risk; however, direct assessment has not been made.

5. The Future

The area-wide project has entered the last three years of its expected duration. A new protocol has been developed to expand the project from the initial demonstration sites to other smaller sites in areas under different land use. Current sites were all established on improved, grazed pastures under cattle production. New demonstration sites will be established on "high value" properties where fire ant control is highly desirable and represents a high economic, environmental, and/or aesthetic value (e.g. parks, poultry farms, hunting clubs, natural areas, military facilities, urban horticulture, etc.). The objective is to expand the area-wide concept to other customers besides cattle farmers and to demonstrate that the concept of using biological controls in combination with toxic bait applications can be used in many different situations. This will take what has been learned from the large-scale area-wide programme on pastures to properties and owners that have a high probability of continuing the fire ant IPM programme after project funding expires. It is expected that these properties will serve as examples for neighbouring property owners, and thus create a knowledge base on fire ant management and biological control that will provide for continuing expansion of interest in fire ant IPM in different regions in the USA.

6. Conclusions

This demonstration has enabled the implementation of IPM for fire ants over large areas, over a sustained length of time, and in diverse areas of the USA. A significant part of fire ant IPM has been the dissemination of self-sustaining parasites and pathogens in the infested areas. For the most part these biological control agents have become established and spread as anticipated or at an even greater rate and population density. In South America, fire ant populations are five to ten times lower than in the USA without the use of pesticides. If introduced natural enemies of the fire ant are half as effective in the USA as in South America, this would lead to a reduction of fire ant populations in the USA by 40-45%. This would significantly reduce pesticide use for fire ant control and diminish the human impact of fire ants, as well as their negative effects on agriculture and the environment. While at this point in time results with biological control agents are not dramatic, they are very encouraging for the long-term future (10-20 years), as additional biological control agents are released.

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