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ABSTRACT

Tropical fish farms provide a prime habitat for the red imported fire ant, *Solenopsis invicta* Buren, which is an invasive, stinging ant that has spread throughout the southern United States. Stings can be a serious health hazard to hypersensitive individuals, and the presence of large populations can interfere with operational activities. The most efficient method of controlling fire ants is the application of ant bait. However, most fish farmers are reluctant to use baits or other chemical methods of control because of the unknown risk to fish. Baited stations in combination with maps generated by geographical information system software were used to estimate locations of fire ant nests. Maps of estimated fire ant locations encompassed from 10 to 100% of actual fire ant nests surveyed when there was a minimum of a single fire ant within a station. Low percentages of overlap between mapped areas and fire ant nests were associated with low nest densities and when higher ant count thresholds were used to indicate positive stations. Ant bait containing the insect growth regulator methoprene was broadcast between ponds, with some unavoidable bait entry into ponds. Fire ant nest densities declined 57% within 4 months. In contrast, nest densities increased 86% in untreated areas. During the summer, fire ant populations declined an average of 68% and increased 110% for treated and untreated areas, respectively. Fire ant nest densities and populations began to increase by December in both treated and untreated areas. No obvious fish mortality related to the ant baiting was noted during the study.

Key Words: *Solenopsis invicta*, aquaculture, GIS application, pest detection, pest control, methoprene, bait, detection.

RESUMEN

Las fincas de peces trópicales proveen un perfecto hábitat para la hormiga de fuego roja importada, *Solenopsis invicta* Buren, la cual es una especie invasora que pica, la cual se ha espacido por todo el sur de los Estados Unidos. Las picaduras pueden ser de gran peligro para la salud de individuos hipersensibles, y la presencia de altas poblaciones pueden interferir con actividades de trabajo. El método mas eficáz para controlar la hormiga de fuego roja importada es la aplicación de cebos para hormigas. Sin embargo, la mayoria de los productores de peces son renuentes para usar los cebos u otros métodos químicos de control por el riesgo desconocido para los peces. Las estaciones de cebo en combinación con mapas generados con programas de computadores de sistemas de información geográficos fueron usados para estimar la localización de los nidos de la hormiga de fuego roja importada. Los mapas de las localidades de la hormiga de fuego roja importada estimadas abarcaron 10 a 100% de los nidos existentes en el muestreo cuando fue un minimo de una sola hormiga de fuego roja dentro de una estación. Los porcentajes bajos de traslapes entre las áreas en los mapas y los nidos de la hormiga de fuego roja fueron asociados con las densidades bajas de nidos y cuando se usaron umbrales de conteo altos de las hormigas para indicar estaciones positivas. El cebo para hormigas que cotiene el regulador de crecimiento de los insectos metoprina fue aplicado entre las lagunas, con alguna entrada inevitable de cebo en la laguna. Las densidades de los nidos de la hormiga de fuego roja bajaron 57% dentro de 4 meses. En contraste, las densidades de los nidos aumentaron 86% en áreas no tratadas. Durante el verano, las poblaciones de la hormiga de fuego roja bajaron un promedio de 68% en áreas tratadas y aumentaron 110% en áreas no tratadas. La densidad y la población de la hormiga de fuego roja empezaron a aumentar por diciembre en ambas áreas tratadas y no tratadas. No se observó mortalidad alguna sobre los peces relacionada con el cebo para las hormigas durante este estudio.

The production of tropical fish accounted for about 43% of the value of Florida aquaculture farm gate sales in 2001 from 160 active growers (Florida Agricultural Statistics Service 2002). Most farms are located in areas with sandy loam soils with a relatively high water table, allowing

for relatively inexpensive pond construction. Ponds are small (0.04 ha), and a typical 0.4 ha (1 acre) of "farm" will have up to eight ponds. Because of the soil type, open water, and high water table, these farms provide an ideal habitat for the red imported fire ant, *Solenopsis invicta* Buren. This ant is an invasive, exotic species from South America, which has spread throughout the southern United States since its inadvertent introduction in the 1930s (Callcott & Collins 1996). It has a painful sting and deaths have been reported in hypersensitive individuals (deShazo et al. 1990, 1999). Fish farmers typically work barefoot and in short pants along the pond banks, and because a fire ant colony can contain over 200,000 individuals (Markin et al. 1973; Tschinkel 1988; Macom & Porter 1996), the probability of being stung is very high. In addition, there are anecdotal reports of mass fish mortality due to the ingestion of fire ants, but studies have failed to confirm this phenomenon (Ferguson 1962; Crance 1965). On most tropical fish farms, fire ants are not controlled because of the fear that insecticides will detrimentally impact fish being raised in ponds (C. A. Watson, pers. obs.).

Effective control options for fire ants in areas where there is a high potential for people to be stung generally involve the use of chemical insecticides. The application of fire ant baits is one of the most efficient methods of control (Lofgren & Weidhass 1972). Baits utilize the natural behavior of ants to forage for food and then share it with colony members. In this manner, insecticides incorporated into baits are spread throughout the colony. Alternative methods of control are to treat individual colonies with an insecticide that must contact the majority of the members of the colony including the queen, or use of nonchemical alternatives such as steam or hot water (Drees et al. 1998; Tschinkel & Howard 1980). However, treating individual colonies is laborious and time consuming in contrast to broadcasting fire ant bait (Barr et al. 1999). Inherent to the integrated pest management approach of controlling insect pests is determining the location and population of the pest (Pedigo 1996). In this study our objectives were to assess the feasibility of (1) using baited monitoring stations to indicate locations of fire ant nests, and (2) broadcasting a fire ant bait to control fire ants on a tropical fish farming facility.

MATERIALS AND METHODS

The study site was located at the University of Florida Tropical Aquaculture Laboratory in Ruskin, FL, which contains 51 ponds on approximately 2.63 ha (6.5 acres). Ponds contained swordtails, *Xiphophorus helleri* L. The banks and paths between ponds, as well as adjacent areas, were visually surveyed for red imported fire ant nests. Each nest was partially opened with a shovel to estimate the amount of adult ants and to search for immature worker caste ants, or brood. The presence of brood indicates that the colony is healthy. In contrast, a colony with no brood or a preponderance of immature reproductive caste ants indicates that the colony is abnormal and declining in vigor. To quantify fire ant populations, a standardized rating system, developed by the U.S. Department of Agriculture, provided a population index that incorporated the estimates of the number of adult ants and the presence or absence of worker caste brood in each nest (Lofgren & Williams 1982). The population indices of all nests in an area were then summed to provide a population index for the area. In addition, all nests containing live ants were counted to provide a density of active nests per area.

Baited monitoring stations also were utilized to determine if they could indicate areas containing active fire ant nests. Stations consisted of two plastic petri dishes (50 mm diameter × 9 mm depth, Gelman Sciences, Ann Arbor, MI). One dish was lined with a cotton cosmetic pad that was moistened with approximately 2 ml of sucrose-based ant attractant (Vail et al. 1999). The other dish was lined with filter paper, and held a toothpick dipped in peanut butter, which served as an oil-based attractant.

Baited monitoring stations were placed at the corners of each pond, and an additional station was placed in the middle of each long side for rectangular ponds or on all sides for square ponds. This resulted in stations being placed at intervals of 8.2 to 9.1 m (27-30 ft) along the upper banks of each pond. Distances between ponds were 3.0 to 6.1 m (10-20 ft). In an area where ponds were not present (control area on the west end), stations were placed at intervals of 7.6 m (25 ft) (Fig. 1). We utilized a total of 230 stations on each sampling date. Stations were set when air temperatures were conducive to ant foraging, i.e., 24.4 to 32.8°C (76-91°F). Fire ants inside stations were counted 30 to 45 min after placement.

Ponds, active nest locations, and monitoring stations were mapped with a geographical positioning system (Trimble Pathfinder Pro-XL, Sunnyvale, CA 94088) which was capable of less than 1 m accuracy. For each sampling date, an inverse distance weighted (IDW) interpolator provided in ArcView GIS software (version 3.2) was used to generate maps that displayed estimated areas of fire ant populations based on fire ant counts at each monitoring station. The IDW interpolator produced maps that were based on the assumption that there would be fewer fire ants in areas that were further away from positive stations. To determine if the maps could be used to indicate the presence of fire ant nests, we calculated the percentages of actual nest locations that were encompassed by these maps.
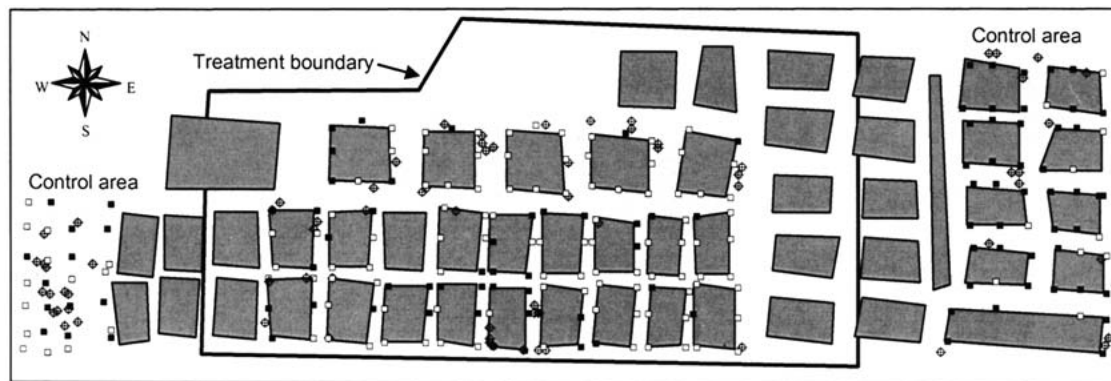
Fig. 1. Study site on March 13, 2000 with monitoring stations which contained fire ants (filled squares) and stations without fire ants (open squares). The lightly shaded area is the map generated from interpolating the ant counts from stations with at least a single fire ant. Actual fire ant nest locations are designated by circles containing a cross. Darkly shaded rectangular areas are the ponds.

The shapes of maps, or estimated areas with fire ants, are affected by the criteria used to define a positive station. Estimated areas with fire ants were mapped with three thresholds for positive stations: 1, 25, and 50 fire ants per station. We hypothesized that by increasing the threshold used to designate a positive station, the percentages of actual nest locations that were encompassed by these maps would decrease because higher thresholds would result in fewer positive stations, and subsequently smaller estimated areas with fire ants. We also hypothesized that the percentages of actual nest locations that were encompassed by maps would be less if fire ant population levels were low. A two-way analysis of variance and Tukey's HSD test (SAS Institute 2001) were used to compare the percentages of nest locations encompassed by maps generated from the three thresholds used to designate positive stations, and between control and treated areas which reflected areas of high and low fire ant populations.

The paths around 32 ponds were treated (Fig. 1) with fire ant bait that contained 0.5% of the insect growth regulator (*S*)-Methoprene (Extinguish™ Professional Fire Ant Bait, Wellmark International, Bensenville, IL 60106). This bait was selected because of the low toxicity of the active ingredient to fish and zooplankton (LC_{50} trout: 760 ppb, bluegill: >370 ppb, and Daphnia 360 ppb). Formulations that contain a much higher percentage of methoprene are used for mosquito control in aquatic ecosystems (e.g., 8.62% methoprene in Altosid®).

Fire ant bait was applied to the paths between ponds with a seed broadcaster (model GT-77 ATV, Herd Seeder Co., Logansport, IN 46947) mounted on an all-terrain vehicle. Bait was applied on March 14, 2000, at a rate of 0.454 kg (1 lb) bait per acre including ponds, which is within the label rate of 0.454 to 0.681 kg (1 to 1.5 lbs) per acre.

However, because bait was broadcast onto paths between ponds, the majority of the bait landed on the paths and banks. This resulted in an application rate of approximately 10.4 kg (23 lbs) of bait per acre if only path surfaces are considered. Two areas encompassing eight ponds were used as untreated controls and were separated from the treated area by approximately 21.3 m (70 ft).

Fire ant populations were determined with the population index and the bait station methods the day before treatment (March 13, 2000) and on 15 (June 29), 22 (August 17), and 39 weeks (December 14) after treatment. Surveys were conducted within a week of moderate to heavy rains to ensure that soil was moist and allowed nest sites to be distinctly visible as mounds. Percent change in fire ant population indices and mound densities from the pretreatment surveys were compared between the treated and control areas.

RESULTS AND DISCUSSION

As the thresholds for positive monitoring stations increased, the percentages of nests that were encompassed by maps that estimated fire ant populations decreased significantly ($F = 27.9$; $df = 2, 18$; $P < 0.001$) (Table 1). However, when fire ant populations were low (≤15 nests/ha) in treated areas, only 10-30% of the nest locations were encompassed by the maps based on the most stringent threshold of 1 ant. In contrast, when fire ant populations were above 35 nests per ha, 79 to 100% of the nests were included in the mapped areas based on the same threshold (Fig. 2). The higher percentages were associated with higher numbers of positive stations (Table 1). Stations with or without ants did not always reflect the presence or absence of nearby nests as there were several observations of fire ant nests adjacent to negative stations (Fig. 1). Perhaps when

TABLE 1. MEAN PERCENTAGES OF *S. invicta* NESTS THAT WERE ENCOMPASSED BY MAPS GENERATED BY INTERPOLA-TION OF POSITIVE MONITORING STATIONS AND THE MEAN NUMBER OF POSITIVE STATIONS (IN PARENTHESES), BASED ON MINIMUM THRESHOLDS OF 1, 25, OR 50 FIRE ANTS PER STATION IN METHOPRENE TREATED AND UN-TREATED CONTROL AREAS AT THE UNIV. OF FLORIDA TROPICAL AQUACULTURE LABORATORY, RUSKIN, FLOR-IDA. MEANS WERE OVER SAMPLING DATES (*n* = 4).

| Area | Threshold No. of *S. invicta* per station | | | Treated vs Control[a] |
	1	25	50	
Treated	52.8 (25.5)	7.2 (10.5)	1.8 (5.0)	20.6a[b]
Control	92.5 (48.3)	43.7 (27.5)	16.6 (16.3)	51.0 b
Comparison among thresholds[c]	72.7 a[d]	25.5 b	9.2 b	

[a]Comparison over all sampling dates and thresholds (*n* =12).
[b]Means followed by a different letter within a column are significantly different by analysis of variance ($F = 25.7; df = 1, 18; P < 0.001$).
[c]Comparisons among thresholds over all sampling dates and areas (n = 8).
[d]Means followed by a different letter within a row are significantly different by Tukey's HSD test ($P < 0.05$).

fire ant nest densities are low, areas may be more accurately mapped by interpolating positive monitoring stations and nest locations that are encountered during the servicing of stations.

The time required to service stations during the March sampling, including the 30-45-min interval to allow ants to forage at stations, was 1.6 person-h. In comparison, a walking survey to flag nest locations required 2.8 person-h. Thus, servicing the bait stations was 43% faster than the walking survey when fire ant populations were relatively high. On the March sampling date when there were 35 and 52 nests per ha (14-21 nests/acre), the single fire ant threshold for a positive station resulted in maps that encompassed over 92% of the nests.

While fish mortality relative to the bait application was not formerly assessed, no obvious mortality was observed during the study (C. A. Watson, pers. obs.). The half-life of technical methoprene in pond water is less than 2 days, and approximately 10 days in soil (EXTOXNET 2001); thus, the accumulation of the active ingredient is probably minimal. The equipment used to apply the bait was calibrated to the recommended label rate. However, the amount of bait actually applied per area of dry land (i.e., excluding water surfaces) was well above the recommended rate. This reflected a typical reality of fire ant bait application, where calibrating commercially available broadcast bait applicators to the very low recommended application rates often is difficult. This difficulty was exacerbated at the fish farm because extremely slow speeds were required to navigate the application equipment through the numerous turns on narrow paths around ponds. Thus, the over-application of fire ant bait represents a problem with bait application that will require improvement.

Fire ant nests per hectare and population indices per hectare one day before bait application were 35 and 564 (14 and 228/acre), respectively,
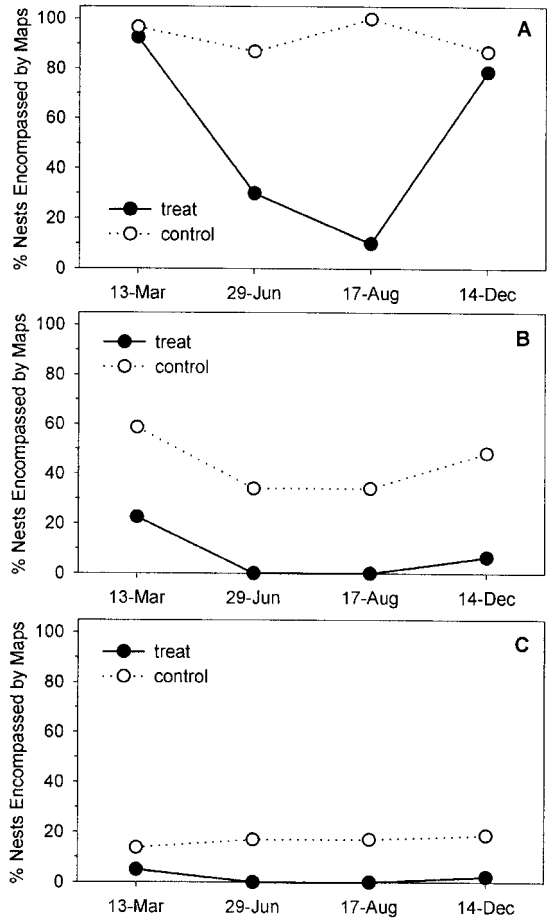

Fig. 2. Percentages of *S. invicta* nests encompassed by maps generated from the interpolation of positive bait stations based on thresholds of (A) 1, (B) 25, or (C) 50 ants between treated and untreated control areas among sampling dates. Treatment was applied on March 14, 2000.

for the treated area, and 52 and 629 (21 and 255/ acre) for the control. Percent reductions of active fire ant nests per hectare in the treated area from the initial survey were 57 and 66% at 15 and 22 weeks after treatment. In contrast, the untreated control areas had 85 and 62% increases in the densities of active fire ant nests. These represent a 167 and 207% difference between the treatment and control for the June and August surveys. Similarly, there were 70 and 67% reductions in population indices in the treated area, and increases of 120 and 99% in the control for the same dates relative to pre-treatment populations. Employees at the facility also perceived a substantial reduction in fire ant stings in treated areas (C. A. Watson, pers. obs.). By the December survey, fire ants had re-infested the treated area beyond the pre-treatment number of active mounds by 83% and the pre-treatment population index by 135% (Table 2).

Methoprene is an insect growth regulator which results in an over production of the reproductive caste and little or no production of adult worker caste ants (Cupp & O'Neal 1973; Vinson & Robeau 1974). Without the worker caste, ant colonies eventually die because food foraging and other colony maintenance functions cease. Because the methoprene concentration in the fire ant bait does not affect adults, colonies will survive until there is sufficient natural attrition of existing adults. This process will generally take 8 to 10 weeks (Drees & Barr 1998) and is consistent with the reduction in fire ants obtained at 15 weeks in this study. Drees & Barr (1998) reported reductions of over 70% at application rates of 1.68 kg per ha, thus the large amount of bait applied in this study probably was not needed to obtain the level of control reported here. The estimated cost of the bait needed for application to the paths in the treatment area in this study was less than $3, based on a cost of $37 per ha at the maximum label rate (1.68 kg/ha).

Low precipitation during the study limited population surveys to irregular intervals dictated by episodes of enough rain to moisten soil and to permit mound building by the ants. When soil is dry, fire ant nests may not be visible, but popula-

tions can still increase. This was evident in the December survey where there was a large increase in fire ant mounds since the August survey. Drees & Barr (1998) reported an increase in fire ant mound densities between 6 and 12 months in methoprene treated field plots. We observed a greater concentration of large colonies in treated areas near a buffer area, which indicated that re-infestation was probably due to migration of mature colonies from the buffer and control areas. If all areas were treated, it would be reasonable to expect a slower reinfestation.

Surveying and mapping active mounds have aided in delimiting areas that require baiting and thus reduced the amount of bait that was applied per year in managed landscapes (Cobb & Cobb 1995). Bait stations could be used on tropical fish farms to delineate areas where fire ants are foraging and could indicate where bait treatments may be effectively applied. Using monitoring stations was faster for us than searching for individual nests, and may be advantageous during dry conditions or in areas with overgrown vegetation, where nests are difficult to see. Counting fire ants at monitoring stations required the ability to distinguish fire ants from other ants. Information on identifying common ants is available in several extension publications (Vail et al. 1994; Drees et al. 2000). Generating interpolated maps based on monitoring stations required investments in GPS equipment and GIS software. Continuing improvements in this technology have made it more affordable and usable. Our use of an IDW interpolator and a 1-ant threshold provided maps that estimated areas which contained over 78% of the fire ant nests when their densities were ≥35 nests per ha (14 nest/acre, Fig. 2a). Evaluations of other interpolators and thresholds may provide more accurate maps over a wider range of nest densities.

In summary, at low fire ant population levels, improvements were needed in estimating nest locations based on the baited monitoring stations. However, when populations were high, the methods utilized in this study detected the presence of most of the fire ant nests. Suppression of fire ants on a tropical fish farm was accomplished with the broad-

TABLE 2. FIRE ANT MOUND DENSITIES AND POPULATION INDICES OF METHOPRENE TREATED AND UNTREATED CONTROL AREAS AT THE UNIV. OF FLORIDA TROPICAL AQUACULTURE LABORATORY, RUSKIN, FLORIDA.

Week[b]	Mounds/ha (% change)		Pop. Index[a]/ha (% change)	
	Treated	Control	Treated	Control
0	35	52	564.1	629.1
15	15 (-57.1)	96 (+84.7)	169.7 (-69.9)	1383.2 (+220.0)
22	12 (-65.7)	84 (+61.5)	188.0 (-66.7)	1253.3 (+99.2)
39	64 (+82.9)	168 (+223.1)	1324.4 (+134.8)	3125.3 (+396.8)

[a]Population index.
[b]Week: 0 = March 13, 15 = June 29, 22 = August 17, 39 = December 14, 2000.

cast application of the methoprene fire ant bait. However, calibration and delivery of bait needs to be more accurate. With improvements, monitoring fire ants at stations and broadcasting fire ant bait has the potential to be an efficient approach of controlling fire ants on tropical fish farms.

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