Distribution and Size of Imported Fire Ant (Hymenoptera: Formicidae) Mounds in Recently Invaded Ball-and-Burlap Nurseries in Tennessee[1]

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Abstract A study was undertaken to describe size and distribution of imported fire ant mounds in south-central Tennessee ball-and-burlap plant nurseries to (1) improve survey and control measures and (2) assess the feasibility of airborne remote sensing for mound detection. Mounds were most numerous along roadsides and road cuts. Mounds in planted areas were larger than mounds along roadsides and road cuts, and mounds in open, grassy areas were of intermediate size (mean above-ground volume = 8.14, 4.36 and 5.32 L, respectively). An examination of mound size distribution in nursery landscapes indicated colony age-structure may not be consistent between landscape-date combinations, and mean mound size was smaller in July 2004 than in October 2003.

Key Words *Solenopsis,* remote sensing, bait

The red imported fire ant (*Solenopsis invicta* Buren), the black imported fire ant (*S. richteri* Forel), and their hybrid pose significant economic threats to the ornamental nursery industry within infested areas of the U.S., as well as health risks to workers in those areas. Farm laborers in fire ant-infested areas are at risk for being stung, which can lead to secondary infections or anaphylaxis. Economic impacts of fire ants in agricultural and urban areas have been documented (Lard et al. 2001). Several agricultural products are subject to quarantine regulations that restrict their movement and/or require specific treatment to eliminate imported fire ants (Title 7, Code of Federal Regulations, Part 301.81). Plant nurseries are economically affected in infested areas due to prescribed insecticidal treatments that must be applied to plant stock before movement from quarantine areas. Ball-and-burlap growers may choose from pre or postharvest approved treatments. Postharvest approved treatments consist of immersing root balls in a chlorpyrifos solution, or watering them with a chlorpyrifos solution over a period of days, whereas preharvest treatments consist of sequential applications of baits and contact insecticides (Callcott 2005). Nurseries may also seek certification as "Imported fire ant (IFA)-free," through a program of

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twice-monthly self-inspection, bait and contact insecticide application, and twice-yearly state or federal inspection (http://www.aphis.usda.gov/ppq/ manuals/domestic/pdf_files/Fire_Ant.pdf (last accessed 28 October 2005).

A study was undertaken in fall 2003 and summer 2004 to characterize imported fire ant populations in two large ball-and-burlap plant nurseries in south-central Tennessee.

Understanding the distribution of fire ant mounds among landscape elements in nursery operations may lead to more effective survey and/or control measures. Information on size and shape of mounds is important for assessing the feasibility of using remote sensing methods for mound detection and optimizing data collection. Finally, basic information on density and age structure of fire ant populations is useful as benchmark data for future studies or control efforts.

Materials and Methods

Study sites. Two ball-and-burlap nurseries located in Franklin and Coffee counties in Tennessee (about 35°4′ 20″ N, 86°13′ 3″ W and 35°23′ 15″ N, 86°8″ 3″ W, respectively) were chosen for this study. Neither nursery had a long history of fire ant infestation. Quarantine compliance agreements were signed in 1999 (Franklin Co.) and 2002 (Coffee Co.) (Steve Powell, TN Department of Agriculture, pers. commun.). Whereas both nurseries used standard insecticidal treatments to comply with quarantine regulations for shipping plants out of the quarantine area, neither had a formal program in place to eliminate fire ant populations over the entire property for IFA-free certification. Nursery personnel sometimes treated individual mounds [usually with Amdro™ (BASF, Research Triangle Park, NC) (hydramethylnon) bait] but were not applying baits by a broadcast method. The area sampled in Franklin Co. was about 65 ha, consisting of rectangular plantings that ranged from about 0.2-1.2 ha. The Coffee Co. site was 220 ha, consisting of rectangular or irregular plantings that ranged from about 0.35-2 ha, and four large (3.8-4.4 ha) fields planted in forage (alfalfa or fescue). Both sites had roads or cuts (grassy strips) between plantings for easy vehicular access. Planted areas consisted primarily of small- to medium-sized shrubs and trees with a few areas of larger trees at the Coffee Co. site.

Aerial surveys. Aerial digital imagery of the sites was obtained from Geodata Airborne Mapping and Measurement, Inc. (Macon, MS) with spatial resolution of 0.15 m (23 October 2003) and 0.22 m (28 July 2004). Four monochrome digital cameras with 10 nm band-pass filters (centered at 450, 550, and 650 nm) and a 20 nm band-pass filter (centered at 850 nm) (GeoVantage™, Inc., Swampscott, MA, USA) were mounted on a Cessna 172 equipped with a GPS antenna and 12-channel receiver, which was integrated with the data collection system. Acceleration and rotation rates for the cameras were monitored with an inertial measurement unit. Proprietary software (GeoVantage™) (Swampscott, MA) was used to georegister and mosaic the images. Images were georegistered to USGS 7.5 min digital elevation models. Horizontal accuracy of the mosaics was ± 3 m (CE90). Imagery was used to classify landscape types within experimental plots, and in a preliminary attempt to classify imported fire ant mounds through photointerpretation.

Ground surveys. A 100 × 100 m grid of sampling points was overlaid onto the images using SoloOffice software (Tripod Data Systems, Corvallis, OR). Each grid point was physically located in the field, and actual sampling points were established as close as possible to the original grid and georeferenced with accuracy of <1 m (rms) using a Starlink Invicta® DGPS receiver (Starlink, Inc., Austin, TX, USA) and a handheld PC with SoloField® software (Tripod Data Systems, Corvallis, OR, USA). A circular plot (0.1 ha) was created around each point and thoroughly searched for imported fire ant mounds. The number of plots differed slightly between sampling dates due to accessibility issues at the time of sampling (e.g., excessively wet ground). Each circular plot contained one or more of the following landscape types: planted area, unpaved road (including the grassy shoulder), unplanted areas predominated by grass foliage, plowed or bare unplanted soil, or completely shaded (mature trees). The rationale for including grassy road shoulders with unpaved road was that the shoulder (about 1-2 m wide) would be easily observed from a vehicle traveling the road. Each mound was measured (long axis, short axis, and height) and its location (landscape type) noted. Mound locations were further characterized within planted areas as between plant rows, within plant rows, or at the base of a plant. Sampling was conducted in November 2003 and July 2004. A photointerpreter outlined the various landscape types within each plot using ArcMap 9.1 (ESRI, Redlands, CA, USA) (Fig. 1). Total area comprising a landscape type within each plot was used to express mound density for each landscape on a per-area basis (mounds ha^{-1}).

Mound area in the plane of the ground was calculated as the area of an ellipse:

$$\text{Area} = \pi * a * b$$

where a is the semimajor axis and b is the semiminor axis. Mound area was used to estimate the spatial resolution that would be necessary to successfully classify fire ant mounds in aerial imagery. Mound size was expressed as above-ground volume of half an ellipsoid, using the equation:

$$\text{Volume} = 2/3 * \pi * a * b * c$$

where a is the semimajor axis, b is the semiminor axis, and c is height from ground

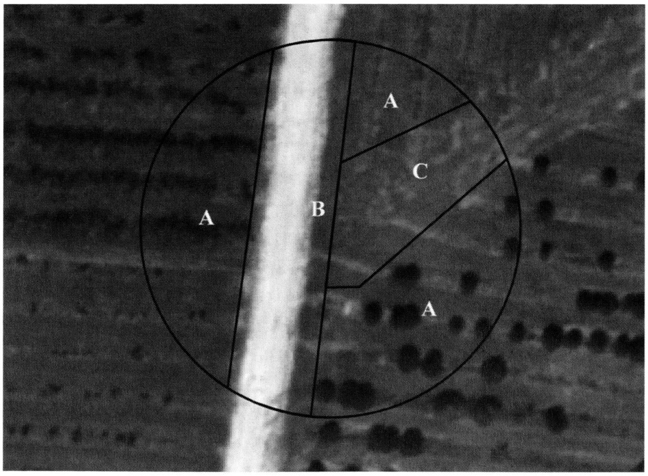

Fig. 1. Typical plot, divided into landscape types: A = planted, B = road / shoulder, C = open, grassy area.

level (Porter et al. 1992). Mound eccentricity was expressed as the ratio of the semiminor axis to semimajor axis.

Weather data were obtained from the National Weather and Climate Center (NWCC) (USDA Natural Resources Conservation Service) SCAN site nearest the nursery sites (Eastview Farm, about 35°08′ N, 86°11′ W). Data consisted of daily rainfall for the month preceding sampling.

Statistical analysis. Data were subjected to statistical analyses to determine possible overall effects of site, date, and landscape type. First, mean distributions were examined to determine normality, and mounds ha^{-1} was converted to a categorical variable (0 mounds ha^{-1}, >0 mounds ha^{-1}) yielding 95% confidence intervals by date and landscape type on the binomial proportion of plots with zero mounds; these data were used to make pairwise comparisons between landscape types, and provided some guidance as to where significant differences might be found. Given the volume of data generated, a more powerful parametric test was performed for the site with the greater number of fire ant mounds. Data (mounds ha^{-1}) were log-transformed and analyzed with Proc Mixed (Littell et al. 1996) to determine the effects of landscape type and sampling date on mound density with plot and plot*landscape type as random effects. Log-transformed means were converted to geometric means and differences of means were converted to ratios. Geometric means were separated using least significant ratios.

Mound characteristics (eccentricity, width, volume above ground, and area in the plane of the ground surface) were compared among landscapes using Proc Mixed with landscape type, sampling date, and their interaction as fixed effects, and plot and plot*landscape type as random effects. Means were separated using least squares means. Mound characteristics were compared among the three possible mound locations within planted areas (within rows, between rows, or at the base of a plant) using Proc Mixed with mound location, sampling date, and their interaction as fixed effects, and plot and plot*mound location as random effects. Means are presented as mean ± SE.

Results and Discussion

The number of plots containing each landscape type and proportion of area sampled comprising each landscape type is summarized in Table 1. The majority of area sampled in both nurseries during both years was planted. More than 50% of the plots also contained some road/roadside area, and a smaller percentage of plots contained grassy, unplanted area. Only two mounds were located in landscape type 4 (plowed area), and none were found in landscape type 5 (mature trees), so these landscapes were not included in the analyses.

Cumulative rainfall totals for the NWCC weather station nearest the sites in the month prior to sampling were 7.2 cm in 2003 and 16.8 cm in 2004. Whereas peer-reviewed, published data on the effects of rainfall on fire ant mound-building activity are not available, it is generally acknowledged that rainfall and other climatic factors can have a dramatic impact on the size and condition of mounds (see Red Imported Fire Ant and Weather Reporting Program, http://fireant.tamu.edu/research/faars/index.html; last accessed 28 October 2005). In particular, during extremely hot and/or dry weather new mound construction may slow or stop, and may resume shortly following rainfall. These short-term effects of weather may complicate ground surveys for mounds. Rainfall totals in the months prior to sampling (about 6.3 cm in October

Table 1. Summary information on landscape types in experimental plots in south-central Tennessee ball-and-burlap plant nurseries

	Landscape occurrence*			
	Franklin County		Coffee County	
Landscape type	2003	2004	2003	2004
Planted	47, 0.527	56, 0.638	211, 0.717	173, 0.682
Road/shoulder	50, 0.205	57, 0.171	134, 0.121	98, 0.107
Grassy open area	28, 0.190	31, 0.187	87, 0.140	83, 0.205
Plowed area	14, 0.078	1, 0.004	14, 0.017	1, 0.003
Shaded/trees	0	0	3, 0.005	4, 0.003

* Number of plots containing landscape, proportion of total area sampled. In Franklin Co., total number of plots sampled was 65 in 2003 and 61 in 2004; in Coffee Co., the total was 227 in 2003 and 187 in 2004.

and 12.6 cm in July) were slightly higher than average rainfall amounts for the area and mounds were generally in good repair.

Very low numbers of mounds at the Coffee Co. site (total of 23 in 2003 and 19 in 2004) made parametric tests impractical, so overall effects of landscape type on mound density were only examined for the Franklin Co. site. Mound density was similar in November 2003 and July 2004 ($F = 3.83$; $df = 1, 102$; $P = 0.0529$) although there was a trend toward higher mound density in November 2003. Landscape type had a significant effect on mound density ($F = 4.28$; $df = 2, 92$; $P = 0.0167$). Mound density in planted areas was lower than the density along roads, but statistically indistinguishable from density in open, grassy areas (Table 2).

Potential effects of landscape type on mound characteristics were investigated by combining data across sites (Table 3). Mounds within planted areas were larger on average than mounds in open, grassy areas or roadside areas ($F = 4.92$; $df = 2, 22$; $P = 0.0172$), and mean mound volume was greater in November 2003 than July 2004 ($F = 19.8$; $df = 1, 133$; $P < 0.0001$). Overall, the mean volume of imported fire ant

Table 2. Geometric means of imported fire ant mound density in landscape types within a south-central Tennessee ball-and-burlap plant nursery.

Effect	Date	Landscape*	Geometric mean ± SE*
Date	Nov. 2003	—	2.52 ± 1.16a
Date	Jul. 2004	—	1.69 ± 1.15a
Landscape	—	Planted	1.46 1.17b
Landscape	—	Road/shoulder	2.81 1.17a
Landscape	—	Open grassy area	2.15 1.13ab

* Mounds ha^{-1}; geometric means within an effect followed by the same letter are not significantly different (least significant ratio, $P > 0.05$).

Table 3. Size and shape characteristics of imported fire ant mounds in three landscape types on two dates in south-central Tennessee ball-and-burlap plant nurseries*

Effect		Mound area* (m^2)	Mound volume (L)	Mound width (cm)	Mound height (cm)
Landscape	Planted	0.17 ± 0.01a	8.14 ± 0.96a	37.97 ± 1.71a	11.70 ± 0.81a
	Road/shoulder	0.15 ± 0.02a	4.36 ± 1.04b	35.56 ± 1.86a	7.91 ± 0.90b
	Open grassy area	0.17 ± 0.02a	5.32 ± 1.58ab	40.87 ± 2.81a	8.57 ± 1.38ab
Date	Nov. 2003	0.20 ± 0.01a	8.42 ± 0.88a	43.24 ± 1.57a	11.17 ± 0.68a
	Jul. 2004	0.12 ± 0.02b	3.46 ± 1.06b	33.03 ± 1.90b	7.62 ± 0.86b

* All data are mean ± SE. Means within an effect followed by the same letter are not significantly different (least squares means, $P > 0.05$).

mounds in nursery planted areas was somewhat lower than mean volume of mounds in northeastern Mississippi pasture, which can exceed 40 L (Vogt et al. 2004). Several factors might be responsible for this difference, alone or in combination, including weather, soil type, disturbance (including insecticide application), and date. Mound area was similar among landscapes ($P > 0.05$) but greater in November 2003 than July 2004 ($F = 19.6$; $df = 1, 133$; $P < 0.0001$). Mound height was greatest in planted areas ($F = 5.51$; $df = 2, 22$; $P = 0.0115$) and greater in November 2003 than July 2004 ($F = 15.09$; $df = 1, 133$; $P = 0.0002$). Mound width was not affected by landscape, but mounds were wider in November 2003 than in July 2004 ($F = 22.61$; $df = 1, 133$; $P < 0.0001$). Within planted areas, 35% of mounds were located within a plant row, 42% were located between plant rows, and 23% were located at the base of a plant. The location of mounds within planted areas did not have a significant effect on mound size and shape characteristics. Mound eccentricity was not affected by landscape or sampling date, and averaged 0.78 ± 0.01.

Distribution of fire ant mound sizes (expressed as above-ground volume) was examined using box-and-whisker plots (Fig. 2). Size distributions were right-skewed in all landscape-date combinations. In November 2003, the skew appeared to be due primarily to clustering of smaller values (small mounds) about the median in road edge landscapes, and due to high values (large mounds) in planted and grassy, open landscapes. The opposite was true in July 2004, when planted and grassy, open landscapes contained a disproportionate number of small mounds. Figure 2 may provide some clues as to the age-structure of imported fire ant populations within the different nursery landscapes. Mound size is clearly related to colony size and biomass (Tschinkel 1993, Macom and Porter 1996). Tschinkel (1993) was able to explain about 89% of the variation in fire ant mound volume using total colony biomass during a year-long study of imported fire ant colonies. Sampling date accounted for only a small percentage of the variation in mound volume, since colony biomass changed with mound volume during the year, being lowest in midsummer. An overall decrease in mound size during summer sampling was apparent in our study. Where clustering of small mounds about the median is apparent (road edges in November 2003, planted areas in July 2004), younger colonies probably predominate. One would need

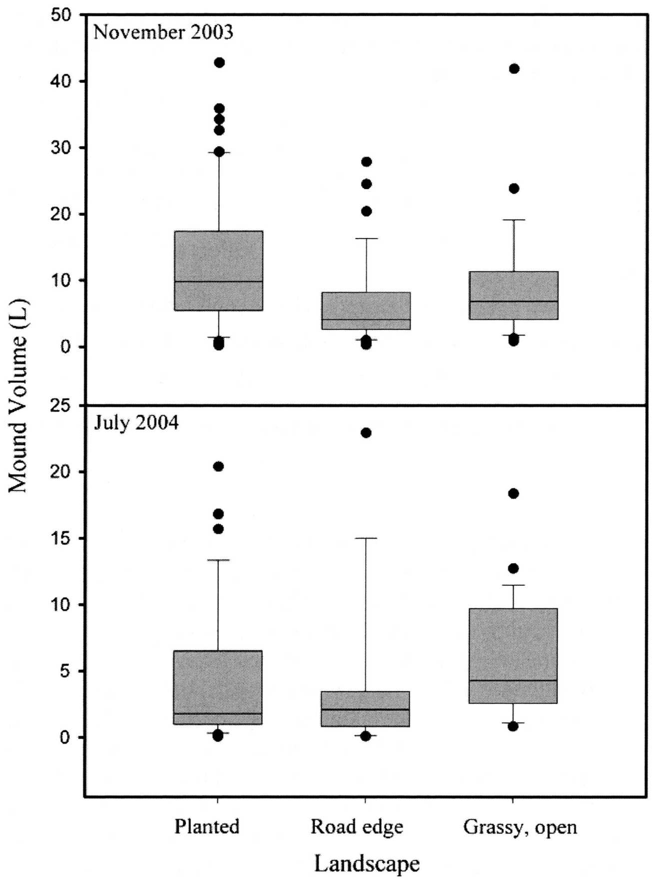

Fig. 2. Box-and-whisker plots of imported fire ant mound sizes (volume in L) in three landscape types within south-central TN ball-and-burlap nurseries.

to examine colony composition in different nursery landscapes to test this hypothesis. Regardless of changes in population age-structure over dates within landscape types, planted areas consistently contained fewer but larger mounds than road edge landscapes.

Disturbance can play a role in fire ant mound distribution among different habitats (Stiles and Jones 1998). In our study, mounds tended to be less abundant but larger in planted areas when compared with roads. Unlike other agroecosystems such as row crops, ball-and-burlap production areas may remain planted for months or years at a time. Planted areas are not undisturbed, however, because they may be regularly mowed or tilled between rows, sprayed, and/or fertilized. Within planted areas over half of the fire ant mounds were located within rows, between or at the base of plants, where physical disturbance would presumably be minimal. Other factors such as shade or resource availability could influence fire ant mound distribution in nurseries.

Current survey methods for imported fire ants recommended for state and federal regulatory officers are included in the USDA Imported Fire Ant Training Manual [available online at http://www.aphis.usda.gov/ppq/manuals/domestic/pdf_files/Fire_Ant.pdf (last accessed 28 October 2005)]. Recommendations for surveying in nursery areas are not specific, but include intense visual surveys supplemented with delimiting surveys using bait or attractant traps. Our findings indicate that persons conducting surveys should not restrict their activities to roads and road cuts in ball-and-burlap nurseries, but should include interior portions of large planted areas. Whereas weather patterns may influence the condition and size of fire ant mounds, overall seasonal trends should be taken into consideration as well due to fluctuations in colony biomass and associated changes in mound size. Summer sampling, regardless of weather conditions, might be less effective than fall or spring sampling. Our study supports this, as mounds were smaller during July sampling in spite of relatively moist soil conditions.

The results of this study also have implications for remote detection of imported fire ant mounds. Target size and spatial resolution are important considerations when planning remote sensing operations. Generally, the spatial resolution of the imagery should be less than half the size of the target in its smallest dimension (Jensen 1996). The width (short axis) of mounds at the sites (Table 3) indicate that spatial resolution $\leq$16.5 and 21.5 cm would be required to detect mounds in November and July, respectively (assuming that sufficient spectral reflectance differences exist between mounds and their surroundings). Other mound attributes such as percent vegetation cover and activity can influence detection in multispectral airborne imagery (Vogt 2004). Preliminary attempts to quantify mounds with aerial imagery of the nursery sites using spatial resolution of 15 cm (October 2003) and 22 cm (July 2004) were not successful (Vogt, unpubl. data). The few mounds that were visible in imagery of the nursery sites occurred in open, grassy areas or along roadsides, and lacked the halo of vigorous vegetation reported in other aerial imagery of mounds (e.g., Green et al. 1977, Vogt 2004). Mean area of mounds at the nursery sites (0.15-0.17 m^2) was generally less than mean area of mounds in a previous study using pasture areas (0.18-0.27 m^2) (Vogt 2004), a difference that might be due to weather, soil type, colony size, date, or a combination of factors. In the latter study, >70% of active imported fire ant mounds were detected using photointerpretation of multispectral airborne imagery collected in May at 10 cm spatial resolution. The relatively heterogeneous environment of ball-and-burlap plant nurseries and the small size of mounds offer challenges for remote detection of imported fire ant mounds. Research to improve detection is ongoing.

This study also yielded information that may be useful for formulating control strategies to reduce imported fire ant density in ball-and-burlap nurseries. Large mounds in relatively protected areas (planted areas) should not be overlooked, especially if a grower is seeking IFA-free certification. When broadcast on the ground, fire ant bait products are usually applied with modified seed spreaders that contain oscillating agitators (rather than spinning agitators) and precalibrated apertures in the metering plate designed to allow the appropriate amount of bait to be gravity-fed to a spreader. Spreaders usually cover a 6.1 m swath, but tall plants or lack of vehicular access within many planted areas in ball-and-burlap nurseries may reduce the effectiveness of such devices. These obstacles may be overcome through aerial bait application, or use of a bait blower that can disperse bait from 15-30 m (see http://agnews.tamu. edu/fireants/stories/baitblower.htm; last accessed 26 September

2005). Growers who are not seeking IFA-free certification must weigh the potential benefits and risks of broadcast bait application; if mound densities are extremely low, they may opt to concentrate solely on treatment of nursery stock prior to shipment. Fire ant bait products are not selective, and avoiding broadcast application may minimize adverse impacts on native ant species, some of which are capable of attacking and destroying young fire ant colonies (Rao and Vinson 2004).

In conclusion, differences exist in the number and size of imported fire ant mounds in different landscape types within ball-and-burlap plant nurseries. The conditions that contribute to these differences have not yet been identified but may include disturbance, resource availability, shade, or other abiotic and/or biotic factors. Future research efforts will attempt to further identify these factors, and characterize the composition of fire ant colonies in nursery landscapes.

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