Statewide Survey of Imported Fire Ant (Hymenoptera: Formicidae) Populations in Tennessee[1]

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Abstract Imported fire ants (*Solenopsis* spp.; Hymenoptera: Formicidae) occupy 54 counties (~5.4 million ha) in Tennessee. To better understand the fire ant species distribution in Tennessee, the state was divided into 16.1 × 16.1 km grids, and a single colony was sampled for cuticular hydrocarbon and venom alkaloid analyses within each grid. A total of 387 samples was processed from which 9 (2.3%), 167 (43.2%), and 211 (54.5%) were identified as red (*Solenopsis invicta* Buren), black (*Solenopsis richteri* Forel), or hybrid (*S. invicta* × *S. richteri*) imported fire ants, respectively. The *S. invicta* was only found near metropolitan Nashville in Davidson and Williamson counties and at one site in Decatur Co. All samples east of Franklin Co. were identified as hybrids. Tennessee counties west of Lincoln were predominantly *S. richteri* (86.5%) as opposed to hybrid (13.0%) and *S. invicta* (0.5%). The exception was Hardin Co., which was predominantly hybrid. Counties containing both hybrid and *S. richteri* (all in the middle and western part of the state) included Bedford, Decatur, Franklin, Giles, Hardeman, Hardin, Haywood, Lawrence, Lincoln, Marshall, Maury, McNairy, Perry, and Wayne. The *S. invicta* samples collected from one Williamson Co. site were determined to be polygyne and infected with the *Solenopsis invicta* virus (genotype SINV-1A). This was the first detection of polygyne imported fire ant in Tennessee. The SINV-1A virus was also a new find at the time of detection, but has been previously reported. The survey results are being used to direct current and future biological control efforts against imported fire ants in Tennessee.

Key Words *Solenopsis invicta*, *Solenopsis richteri*, hybrid, distribution, polygyne

Imported fire ants (*Solenopsis* spp.; Hymenoptera: Formicidae) are serious pests now infesting about 136 million ha in the United States (USDA-APHIS 2007a). There are numerous impacts associated with imported fire ants, including health threat to humans and livestock, recreational nuisance, damage to property (e.g., electrical systems, roads), direct crop damage, disruption of natural ecosystems, legal issues, costly insecticide treatments, and quarantines of commodities like nursery stock

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(Vinson 1997, Gotelli and Arnett 2000, Haun 2000, Lard et al. 2001, Wojcik et al. 2001, Haun and Powell 2006). The first report of imported fire ants in the state of Tennessee was an accidental introduction in 1948 in Shelby Co., but the actual population front did not enter the state until 1987 in Hardin Co. (TDA 2008). Imported fire ants are continuing to expand their range northward in the U.S. and now infest over 5.4 million ha in Tennessee (~50.2% of the state), which represents 40 complete and 14 partial counties (TDA 2008).

Two species of imported fire ant have been introduced into the United States with the black (*Solenopsis richteri* Forel) arriving first around 1918 followed by the red (*Solenopsis invicta* Buren) around 1930 (Callcott and Collins 1996). Both species likely entered the U.S. near the Port of Mobile, AL, in shipping dunnage (Vinson 1997). The subsequent range expansion of imported fire ants across the southern U.S. was facilitated by their rapid natural dispersal mechanism (i.e., mating flights) and human assisted transport. A number of regulated articles can easily transport imported fire ants, including nursery plants, sod, soil or unwashed soil moving equipment, and hay stored in contact with the ground (USDA-APHIS 2007a, b). The Federal Imported Fire Ant Quarantine was not enacted until 1958 (USDA-APHIS 2007b), which allowed imported fire ant populations to be disseminated for almost 20 yr by articles that are now regulated. As a result, *S. invicta* and *S. richteri* were dispersed throughout the southern U.S. resulting in a complex mixture of the two species. There is a general lack of data concerning the historical distributions of *S. invicta* and *S. richteri* over the past 90 yr, but it appears that *S. invicta* has displaced *S. richteri* to the northern areas of the current imported fire ant range (Vander Meer et al. 1985, Callcott and Collins 1996). It is now well established that *S. invicta* and *S. richteri* alates can mate to form a reproductively functional hybrid (Vander Meer et al. 1985, Ross et al. 1987, Vander Meer and Lofgren 1989).

Several studies indicate imported fire ant populations in northwestern Georgia and northeastern Alabama are mostly hybrid, whereas populations in northwestern Alabama are a mixture of hybrid and *S. richteri* with a predominance of *S. richteri* (Diffie et al. 1988, Gardner et al. 2008, Graham et al. 2005, 2006, Streett et al. 2006). Taber (2000) indicated the range of *S. richteri* is restricted to northwestern Alabama and northeastern Mississippi reaching to the Tennessee border, whereas *S. invicta* occupies most of the southern U.S. below Tennessee. However, *S. invicta* was almost nonexistent in samples collected from northern areas of Alabama, Georgia, and Mississippi in other studies. It is likely the distribution of imported fire ant species and their hybrid in Tennessee will be similar to fire ant distributions in adjacent states. Callcott and Collins (1996) citing a personal communication from an USDA-APHIS survey performed in southern Tennessee reported 4 counties with hybrid imported fire ant and 8 counties with *S. richteri*, but did not indicate the specific county locations.

It is important to determine the distribution of imported fire ant species and their hybrid in Tennessee to improve the success rate of on-going classical biological control programs. The USDA has an active program to establish imported fire ant parasitoids, decapitating flies (*Pseudacteon* spp.; Diptera: Phoridae), throughout the southern U.S. (Callcott et al. 2007). Many *Pseudacteon* species exhibit distinct preferences for *Solenopsis* species (Gilbert and Morrison 1997) or host-specific characters like body size (Porter 1998, Orr et al. 1997). Hybrid and *S. richteri* colonies in Tennessee routinely have larger individuals than *S. invicta* colonies (James et al. 2002), and some phorid species prefer larger workers (Porter 1998, Orr et al. 1997). In addition, many phorid species have distinct biotypes that exhibit preferences for different imported fire ant species, often with modifications to the ovipositor shape that are presumed to be host-specific (Porter and Pesquero 2001). For example, *Pseudacteon curvatus* Borgmeier, Los Flores biotype, exhibits a greater preference for hybrid and *S. richteri* than *S. invicta* (Porter and Briano 2000). A number of phorid species and biotypes have been released in Tennessee with mixed establishment results (Parkman et al. 2005), so a statewide survey of imported fire ant species and their hybrid may facilitate the success of future releases. The objective of this study was to determine the distribution of imported fire ant species and their hybrid in Tennessee by sampling colonies and identifying the ants with cuticular hydrocarbon and venom alkaloid analyses.

Materials and Methods

Fire ant sample collection and preparation. Tennessee counties that were partially or completely infested with imported fire ants during 2004 were divided into 16.1 × 16.1 km grids. Within each grid, personnel from Tennessee State University (TSU) and the Tennessee Department of Agriculture (TDA) searched until one fire ant colony was located for sampling. The colony was sampled by inserting a glass scintillation vial (20 ml) into the mound to collect a minimum of 30 worker ants. Each sample site was marked with global positioning unit (GPS). Sites sampled by TDA personnel were marked with a Garmin eTrex® GPS (Garmin International, Inc., Olathe, KS) or a Magellan® GPS 315 (Magellan Navigation, Inc., San Dimas, CA). Sites sampled by TSU personnel were marked with a either a LandMark Sytems© RT-INW-I submeter WAAS DGPS running SoloField™ CE3.2 or a LandMark Systems© CSI submeter series with a Tripod Data Systems™ Recon running SoloField™ CE (LandMark Systems, Tallahassee, FL). Other collection information including the date, location details, and collector were also obtained for each sample site. Samples were frozen by collectors and then mailed to the TSU Otis L. Floyd Nursery Research Center, McMinnville, TN (TSU-NRC). At the TSU-NRC, samples were stored in a freezer until they could be processed in the same general time frame. A minimum of 25 worker ants were transferred to a new 20 ml scintillation vial with just enough hexane GC Resolv (85% minimum n-hexane, GC) (Fisher Scientific, Pittsburgh, PA) to cover the ants. The ants were soaked in hexane for 20-24 h. Hexane was then removed with a glass pipette and transferred to an empty 20-ml glass scintillation vial. The hexane was allowed to evaporate from the vial and then the empty vial was capped. Samples obtained during 2004 and 2005 ($n = 235$) were shipped to the USDA Center for Medical Agricultural and Veterinary Entomology, Gainesville, FL (CMAVE) for cuticular hydrocarbon and venom alkaloid analyses. Samples obtained after May 2005 ($n = 152$) were processed at the TSU-NRC.

Chemical analysis. At the CMAVE, the evaporated worker ant washes were reconstituted in 0.5 ml hexane, vortexed for 2–3 sec, and allowed to stand at room temperature for at least 2 h before analysis. Venom alkaloids and cuticular hydrocarbons were the major components in the wash, and both classes of compound could be analyzed in a single gas chromatography run. Samples were processed using an Agilent 6890N Network Gas Chromatograph System (Palo Alto, CA). The Agilent System was equipped with a split-splitless injector, a flame ionization detector, and a DB-1 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific Inc., Folsom, CA). The injector and detector were set at 300°C, and the oven temperature was programmed from 150° to 285°C at 10°/min and then held at 285°C for 4 min. Hydrogen served as the carrier gas and nitrogen was used as the makeup gas. Data were analyzed using Agilent Technologies GC Chemstation G2071AA A.10.01 (Agilent Technologies, Palo Alto). Peak retention times were compared with standard venom alkaloids and cuticular hydrocarbons from *S. invicta* and *S. richteri* (Ross et al. 1987). If there was ambiguity in peak assignment, then mass spectra were obtained on an Agilent 5973 Network Mass Selective Detector US10480853 using Agilent 6890N Network Gas Chromatography System US10124023. The injector was set at 300°C and the oven temperature was programmed from 100° to 285°C at 10°/min, and then held at 285°C for 10 min with the transfer line at 285°C. Helium was used as the carrier gas. Data were analyzed using Agilent Enhanced GC/MS Chemstation software G1701DA version D.00.00.38.

Some imported fire ant samples were submitted to the TSU-NRC during 2004 and 2005 without GPS coordinates or with coordinates that did not plot in the appropriate quadrant grid. County samples submitted without GPS data ($n = 49$) included Bradley ($n = 7$), Hamilton ($n = 10$), Maury ($n = 2$), McMinn ($n = 9$), Meigs ($n = 6$), Polk ($n = 8$), Rhea ($n = 6$), and Wayne ($n = 1$) counties. County samples with GPS coordinates not plotting in the correct quadrant ($n = 21$) included Coffee ($n = 1$), Franklin ($n = 1$), Hardeman ($n = 6$), Hardin ($n = 4$), Haywood ($n = 1$), Lawrence ($n = 1$), McNairy ($n = 6$), and Meigs ($n = 1$) counties. The imported fire ant samples from these sites were processed for cuticular hydrocarbon and venom alkaloid analyses at the CMAVE and the results added to the total counts for the state, but the exact site locations for these samples were undetermined. All of these problematic samples were resampled during 2007 and 2008 to obtain accurate GPS coordinates and the new samples were processed for cuticular hydrocarbons and venom alkaloids at the TSU-NRC. In addition, TSU-NRC personnel collected additional samples during 2007 and 2008 to compensate for any large gaps within counties that were created by surveyors collecting points too close to the edges of adjoining quadrants. Only new sample points and previous points that were correctly collected were used to prepare the species distribution map (Fig. 1).

All samples collected after May 2005 were processed as before for venom alkaloids and cuticular hydrocarbons, except the analysis was performed at the TSU-NRC using a Shimadzu 17-A gas chromatograph (Shimadzu Instruments Inc., Columbia, MD) equipped with a flame ionization detector. The fused silica 30 m × 0.25 mm i.d.

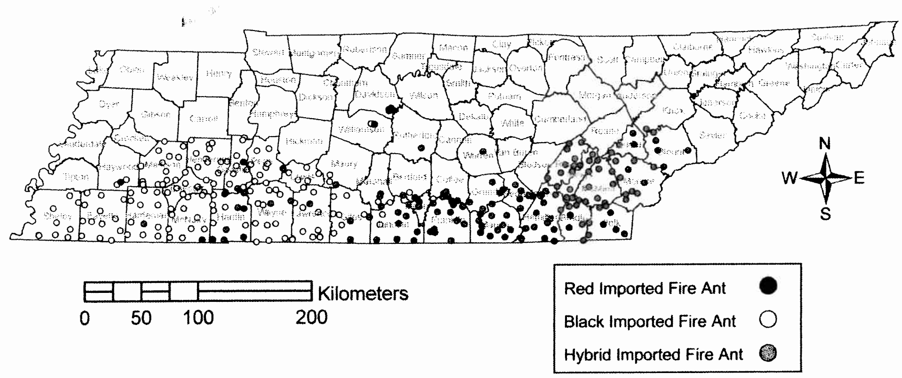

Fig. 1. Site locations for *S. invicta* and *S. richteri* and their hybrid in Tennessee.

DB-1 capillary column (Agilent Technologies) was programmed from 80° to 275°C at 15°C/min and then held at 275°C for 7 min.

One imported fire ant site in Williamson Co. was a suspected polygyne site, so samples were also collected in 95% ethyl alcohol from 3 colonies during July 2005 and sent to CMAVE for polygyne testing as described by Valles and Porter (2003). The CMAVE facility also performed a test for *Solenopsis invicta* virus-1 on these Williamson Co. ant samples (Valles and Strong 2005). The distribution of imported fire ant species and their hybrid were mapped using ArcGIS version 9.1 (ESRI Inc., Redlands, CA) (Fig. 1).

Results and Discussion

A total of 316 imported fire ant samples from 38 infested counties in Tennessee was received with correct global positioning (GPS) coordinates and were used for mapping imported fire ant distributions within Tennessee (Fig. 1). Among these samples, 160 (50.6%), 147 (46.5%), and 9 (2.8%) were identified as hybrid, *S. richteri*, or *S. invicta*, respectively. All samples east of Franklin Co. were exclusively hybrids in this survey. The majority of counties in middle and west Tennessee were a mixture of predominantly *S. richteri* with some hybrid. Exceptions were Moore Co., which only had hybrid imported fire ant recovered and Chester, Fayette, Henderson, Lewis, Madison, and Shelby counties, which only had *S. richteri* recovered. Hardin was the only western county with a predominance of hybrid imported fire ant. The only samples in this survey identified as *S. invicta* were from Decatur (n = 1), Williamson (n = 2), and Davidson (n = 6) counties. One sample from Williamson Co. also was identified as *S. richteri*. Samples from Davidson, Rutherford, Warren, and Williamson counties were isolated infestations outside of the Federal Imported Fire Ant Quarantine at the time they were collected (TDA 2008).

The *S. invicta* site in Williamson Co. at GPS coordinate N35.932392 W86.801207 was polygyne and infected with the SINV-1A virus (Valles et al. 2007). This is the first report of polygyne imported fire ant in Tennessee. The SINV-1A virus was also a new state report with details provided in Valles et al. (2007).

In addition to samples received with GPS coordinates that could be mapped (Fig. 1), some samples were received without GPS information or with erroneous coordinates within the county. These samples were also processed for species identification, but could not be mapped. When combined with the points that were mapped, this survey found a total of 9 (2.3%), 167 (43.2%), and 211 (54.5%) *S. invicta*, *S. richteri*, and hybrid imported fire ants, respectively. All combined samples (both mapped and unmapped) from east Tennessee were hybrids.

The distribution of imported fire ants in Tennessee is indicative of ant distributions in states below Tennessee. The northwestern tier of Georgia and the northeastern tier of Alabama are predominantly hybrids (Diffie et al. 1988, Gardner et al. 2008, Graham et al. 2005, 2006), and are adjacent to Tennessee areas that are exclusively hybrid. Likewise, the northeastern tier of Mississippi appears to be predominantly *S. richteri* with an occasional hybrid sample (Streett et al. 2006), which also corresponds to portions of west Tennessee that were predominantly *S. richteri* with periodic hybrid samples. These findings suggest imported fire ant distributions in Tennessee are the result of natural dispersal from adjoining states. The likely exceptions are the *S. invicta* sites identified in Davidson, Decatur, and Williamson counties. The Tennessee locations with *S. invicta* are hundreds of kilometers from the nearest *S. invicta* population in bordering states (Diffie et al. 1988, Gardner et al. 2008). In addition, the *S. invicta* sites in Davidson and Williamson counties are isolated north of the primary imported fire ant front in Tennessee. Consequently, it is likely *S. invicta* populations were introduced artificially by human-aided transport. Unfortunately, one of the *S. invicta* sites in Williamson Co. was determined to be the polygyne reproductive form. The site was sampled for polygyne status because the mound densities were atypically high for Tennessee, and the worker size was uniformly small. It is likely polygyne colonies will have a greater impact on Tennessee citizens because polygyne sites can have twice as many mounds and workers per unit area as monogyne sites (Porter et al. 1991, Macom and Porter 1996).

In terms of *S. invicta* establishment in North America, the Davidson Co. latitude (i.e., 36°10′00"N) has been defined as "possible" to "undemonstrated" (i.e., sites with weather conditions that have no record of success for *S. invicta*) by different range expansion models (Korzukhin et al. 2001, Morrison et al. 2005). The Davidson Co. sample sites were the most northern points surveyed and were all in the metropolitan Nashville area. The Davidson Co. site occurs between the minimum yearly temperature lines of −12.3 and −17.8°C, the latter being an arbitrary range limit for *S. invicta* proposed by Vinson (1997). Field observations indicate the *S. invicta* populations in Davidson and Williamson counties are extensive and indicative of a well-established and expanding population. The Tennessee Department of Agriculture has been attempting to eradicate imported fire ants from Davidson and Williamson counties for approx. 14 yr (G. Haun, pers. comm.), further supporting that *S. invicta* can occupy latitudes as far north as Nashville.

Sample site elevations for hybrid, *S. richteri*, and *S. invicta* in this study ranged from 190-612, 74-317, and 160-260 m, respectively. East Tennessee sites, all of which were exclusively infested with hybrids, are more mountainous than other sample sites. Hybrids were collected at 600-612 m (n = 2), 500-599 m (n = 13), 400-499 m (n = 2), 300-399 m (n = 24), 200-299 m (n = 83), and 100-199 m (n = 3), respectively. The *S. richteri* were collected at 300-317 m (n = 3), 200-299 m (n = 33), 100-199 m (n = 57), and 0-99 m (n = 8), respectively. The *S. invicta* were collected at 200-260 m (n = 2), and 100-199 m (n = 5), respectively. Cumberland Plateau sites with hybrids are undoubtedly colder during winter months than lower elevation sites. Like *S. invicta* populations in Davidson Co., hybrid populations appear to be well established on the Cumberland Plateau. Hybrids were more resistant to extended cold periods than *S. invicta* or *S. richteri* in one study, and this finding was proposed as one explanation for the distribution of imported fire ants in the U.S. (James et al. 2002). A greater percentage of hybrids occurred at higher elevations than *S. richteri* or *S. invicta* in our study, further supporting the theory that hybrids are more cold tolerant than the two parent species.

The major advantage offered by this statewide survey is guidance for future biological control releases in Tennessee. The information obtained has already been used to direct the release of *P. tricuspis* in Davidson Co. during 2006 and *P. curvatus* Formosan biotype in Williamson Co. during 2007. Adult *P. tricuspis* have not been recovered at other release sites in Tennessee (Parkman et al. 2005), and it is likely past releases may have failed because adult flies were not matched optimally with their imported fire ant host. One limitation of our survey was that a single imported fire ant colony was sampled from a large survey grid (i.e., ~259 km^2), which means all counties had substantial nonsampled areas. The goal of this survey was to provide a snapshot view of the imported fire ant distributions in the entire state; more refined sampling may be needed in the future if specific delineations are desired for individual counties. For example, the Vail and Parkman (2008) study found hybrid and *S. richteri* in Fayette, Coffee, and Rutherford counties, but our study only found *S. richteri* in Fayette and hybrids in Coffee and Rutherford counties. Likewise, our study found hybrid and *S. richteri* in Maury Co. and hybrid, *S. invicta*, and *S. richteri* in Decatur Co., but the Vail and Parkman (2008) study found only *S. richteri* in these counties. Lastly, our study only recovered *S. richteri* in Shelby Co., but Vail and Parkman (2008) recovered hybrid, *S. invicta*, and *S. richteri* from Shelby Co. The *S. invicta* has also been found in Knox Co. (Vail and Parkman 2008) and recently at another site in Rutherford Co. (S. Ochieng, unpubl. data). It appears the distribution of *S. invicta* in Tennessee, with the exception of the single site in Decatur Co., coincides with the major metropolitan areas of Knoxville, Memphis, and Nashville. Therefore, *S. invicta* are probably being moved with human commerce into these metropolitan areas, which are substantially north of the primary *S. invicta* populations in the southern United States. Although western Tennessee counties had a mixture of hybrids and *S. richteri*, samples west of Lincoln Co. were predominantly *S. richteri* (86.5%) as opposed to hybrid (13.0%) and *S. invicta* (0.5%). All samples collected east of Franklin Co. were hybrids. The imported fire ant populations in Tennessee are expanding northward so rapidly that there are now multiple imported fire ant sites not represented in this study (TDA 2008). The imported fire ant compositions at most nonsampled sites will probably reflect the species compositions of the adjacent sampled areas. It is anticipated this statewide imported fire ant survey will improve the success rate of future phorid releases and enhance the establishment of all biological control efforts in Tennessee.

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