Biodiversity of Fungi in Red Imported Fire Ant (Hymenoptera: Formicidae) Mounds

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ABSTRACT The red imported fire ant, *Solenopsis invicta* Buren, became established in North America more than 70 yr ago, and it currently occupies most of the southeastern United States. Fire ants change the physical and chemical components of soil, which likely influence soil fungi in ant mounds. To determine the effects of fire ants on soil fungi, we sampled soil from fire ant mounds and the surrounding nonmound soil. In addition, we sampled soil from the nests of the native ant *Aphaenogaster texana carolinensis* Wheeler. We found that both fire ant mounds and native ant nests had greater fungal abundance but lower species richness and diversity than nonmound soil. Fire ant mounds contained 19 times more colony forming units (cfu g^{-1}) than adjacent soil; however, nonmound soil had more than twice the number of fungal species. Two species (*Papulaspora byssina* Hotson and *Penicillium janthinellum* Biourge) made up the majority (54.5 and 19.2% relative colony frequencies, respectively) of fungi in fire ant mounds. These high proportions of limited numbers of fungal species in fire ant mounds indicate that only some species are tolerant to and thrive in mound conditions. Alternatively, fire ants might not selectively remove these fungi from their mounds. Given the high densities of fire ants and their frequent mound movements, changes in soil fungal communities might have lasting impacts on soil conditions. In addition, we suggest that differences between fungal communities in soil from native and non-native ant colonies might indirectly influence ant-mediated seed dispersal by affecting seedling survival.

KEY WORDS *Solenopsis, Aphaenogaster*, seed dispersal, fungi, *Papulaspora*

THE RED IMPORTED fire ant, *Solenopsis invicta* Buren, was established in North America in the 1930s (Lofgren 1986), and currently occupies most of the southeastern United States (Calcott and Collins 1996). Fire ants can alter ecological properties of their environments following invasion. Biotically, fire ants can negatively affect vertebrate (Allen et al. 1994), invertebrate (Porter and Savignano 1990), and yeast (Ba et al. 2000) populations. Abiotically, fire ants also may change the physical and chemical properties of soil. For example, through tunnel excavations and mound construction, subsoil is aerated as it is brought to the ground surface (Hays 1959). In addition, occupation by fire ants can change soil pH (Herzog et al. 1976, Blust et al. 1982), increase phosphorus and potassium levels (Blust et al. 1982), and reduce organic matter (Herzog et al. 1976, Blust et al. 1982). These changes in soil conditions will likely influence the microbiota found within fire ant mounds.

Ants employ several tactics as defenses against bacteria and fungi. Some ants physically remove fungi and spores from their bodies, other nestmates, and nest chambers (Hölldobler and Wilson 1990). Other ants can use chemical defense mechanisms. For example, fire ants have venom that contains alkaloids that decrease conidial germination by entomopathogenic fungi (Storey et al. 1991). Secretions from the metapleural gland of the ant *Myrmecia nigriscapa* Roger significantly suppress both mycelial growth (Beattie et al. 1985) and spore germination of soil fungi (Beattie et al. 1986). Leaf cutter ants that culture fungi use antibiotics from actinomycetes to suppress pathogenic fungi (Currie et al. 1999). Furthermore, secretions of alarm pheromones can inhibit fungal growth (Cole et al. 1975). These mechanical and chemical defenses conceivably influence fungal populations in ant mounds.

Given that fire ants are expanding their range, there is growing concern that their presence will have negative ecological consequences. The search for biological controls of fire ants has prompted investigations on the mycological components of fire ant colonies (Jouvenaz et al. 1977, Jouvenaz and Kimbrough 1991); however, in these studies searches were limited to endoparasites. Here we have investigated nonyeast fungi found within fire ant mounds. Our objective was to compare the fungal species richness, diversity, and composition in fire ant mounds, nonmound soil, and in

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Table 1. Fungal species richness, diversity, evenness, and number of colony forming units per gram of dry weight soil (cfu g^{-1}) in five fire ant (*Solenopsis invicta*) mounds, five non-mound soil locations, and five *Aphaenogaster texana carolinensis* nests in Clemson, SC, in 2001

	Species richness	Diversity (H')	Evenness (E)	cfu g^{-1}
Fire ant mounds	14	1.6 (0.114)a	0.61 (0.037)a	503,390 (155,239)a
Non-mound soil	29	3.1 (0.141)b	0.92 (0.061)b	25,995 (4,261)b
Aphaenogaster nests	19	2.5 (0.155)b	0.85 (0.045)b	227,052 (40,726)ab

Shannon-Weaver formulas were used to calculate diversity (H') and evenness (E) indexes. Numbers in parentheses are standard errors. Within-column values with the same letter are not significantly different ($P > 0.05$).

nests of the native ant *Aphaenogaster texana carolinensis* Wheeler (N20 of Umphrey 1996).

Materials and Methods

Soil from *A. texana carolinensis* nests, fire ant mounds, and nonmound areas was sampled on 30 March 2001 from two locations near Clemson, SC. Voucher specimens of *A. texana carolinensis* and *S. invicta* were deposited in the Clemson University Arthropod Collection. Soil and leaf litter from *Aphaenogaster* nests were collected from an eastern deciduous forest in the Clemson Experimental Forest devoid of *S. invicta* mounds. *Aphaenogaster* nest entrances were located by following foragers returning from tuna baits. In the second site, we sampled soil from both active fire ant mounds and nonmound areas (>1.0 m from mounds) in a dairy-cow pasture. Nonmound soil had no evidence of past occupation by fire ants. This pasture contained an average density of 37 fire ant mounds per ha. To minimize disturbance of ant colonies, we collected soil at sunrise when air temperature was 8°C. Worker and brood were not present at the top of the mound when we collected soil samples. For each soil type (*Aphaenogaster* nest, fire ant mound, and nonmound), we used a soil core sampler (5 cm) to obtain five samples of the first 8–10 cm of top soil. The soil sampler was rinsed in 25% bleach (6.0% NaOCl) between samples. Each sample was placed in a plastic bag, sealed, transported in an ice cooler, and returned to the laboratory. All ants and brood that remained in the samples were removed with sterile forceps, and the soil was refrigerated overnight at 4°C.

For each sample, 10 g of mixed soil were added to 90 ml of deionized water and stirred. From this mixture, 10-fold serial dilutions were prepared. Upon obtaining a final dilution of 10^{-3} for each sample, 1.0 ml of the soil suspension was pipetted onto the surface of a rose bengal agar plate (100 by 15 mm). Rose bengal agar media reduces bacterial growth and limits the growth of invasive fungal species (Jarvis 1973). Agar plates were incubated at room temperature (≈25°C) for 4 d, and an average number of colony-forming units (cfu) for all three soil types was calculated. From each plate, 20 randomly selected isolates were transferred to potato dextrose agar slants, yielding a total of 100 fungal colonies for each of the three soil types. These fungal isolates were used to calculate relative sample frequency (% of samples with fungal species), relative colony frequency (percentage of total fungal colonies present), Shannon-Weaver diversity (H') and evenness (E) indexes (Shannon and Weaver 1949), and coefficients of similarity in each soil type. Isolates that failed to grow or were identified as actinomycetes or yeasts were not included in the analyses. We obtained wet and dry (oven dried at 105°C for 72 h) (Winegardner 1996) weights of ≈5 g soil from each of the remaining soil samples to quantify the number of colony-forming units (cfu g^{-1}). Average number of cfu g^{-1} and Shannon-Weaver indexes were analyzed by a one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test where warranted (SAS Institute 1999).

One fungus, *Papulaspora byssina* Hotson (=anamorph of *Myriococcum praecox* Fr.) growing on agar slants, was presented to five fire ant colonies. Each colony was presented one agar slant. After 1 h, the numbers of fire ants foraging on each slant were recorded. In addition, we simultaneously presented uninoculated agar slants to five additional fire ant colonies to determine if fire ants preferentially forage on *P. byssina.* Total numbers of ants foraging were compared with a one-way ANOVA (SAS Institute 1999).

Results

We found that the mean cfu varied for the three soil types. Fire ant mounds had a significantly ($F_{2,27} = 6.69$, df = 2, $P = 0.004$) higher number (mean = 415, SE = 128) of cfu than adjacent nonmound soil (mean = 22, SE = 3.78), but no significant difference existed between cfu in fire ant mounds and *Aphaenogaster* nests (mean = 169, SE = 30.3). Based on calculated cfu g^{-1}, fire ant mounds had more than twice the number of fungi as *Aphaenogaster* nests (227,052), but these differences were not significant ($P > 0.05$) (Table 1). Fire ant mounds had >19 times the number of cfu g^{-1} (503,390) than did nonmound soil (25,995) (Table 1). However, most of the fungi in fire ant mounds consisted of only two species, *Papulaspora byssina* Hotson (55%) and *Penicillium janthinellum* Biourge (19%) (Table 2). We found that fire ants might not be harvesting *P. byssina* for consumption because there were significantly ($F_{1,8} = 8.27$, df = 1, $P = 0.020$) more ants foraging on uninoculated agar than agar with *P. byssina. Papulaspora byssina* was found in low numbers (2.4%) in *Aphaenogaster* nests; however nonmound soil lacked this fungus (Table 2). *Penicillium janthinellum* was found in the nonmound soil, but its relative colony frequency was only 2.6% (Table 2).

Table 2. Relative sample frequency (% of samples with fungal species) and relative colony frequency (% of total fungal colonies present) of fungal species found in soil collected in Clemson, SC, in March 2001 from five red imported fire ant (*Solenopsis invicta*) mounds, five non-mound soil locations, and five *Aphaenogaster texana carolinensis* nests

Fungal species	*S. invicta*		Non-mound soil		*A. texana carolinensis*	
	Sample frequency	Colony frequency	Sample frequency	Colony frequency	Sample frequency	Colony frequency
Absidia cylindrospora Hagem.	—	—	—	—	20	1.2
Absidia spinosa Lendn	—	—	40	2.6	40	2.4
Aspergillus flavipes (Bainier and Sartory) Thom and Church	—	—	20	1.3	—	—
Cladosporium cladosporioides (Fresen.) deVries	—	—	40	3.8	—	—
Fusarium sp. 1 Link	—	—	80	9.0	—	—
Fusarium sp. 2	40	2.0	—	—	—	—
Fusarium sp. 3	40	3.0	40	5.1	—	—
Fusarium sp. 4	—	—	—	—	60	3.6
Fusarium sp. 5	—	—	40	2.6	—	—
Fusarium sp. 6	—	—	20	1.3	—	—
Fusarium sp. 7	—	—	20	1.3	—	—
Fusarium culmorum (W. G. Smi.) Sacc.	—	—	40	2.6	—	—
Fusarium dimerum Penz	—	—	20	2.6	—	—
Fusarium merismoides Corda	40	2.0	—	—	—	—
Fusarium oxysporum Fr.	—	—	60	15.4	—	—
Gliocladium sp. 1 Corda	—	—	20	1.3	—	—
Gliocladium sp. 2	—	—	40	2.6	—	—
Gongronella butleri (Lendn.) Peyronel and Dal Vesco	—	—	20	3.8	40	2.4
Mortierella sp. 1 Coem	—	—	—	—	60	8.3
Mortierella sp. 2	—	—	20	1.3	100	10.7
Mortierella sp. 3	—	—	—	—	40	3.6
Mucor hiemalis Wehmer	20	4.0	—	—	—	—
Mycelia sterile sp. 1	20	1.0	—	—	60	4.8
Mycelia sterile sp. 2	—	—	40	3.8	—	—
Papulaspora byssina Hotson	100	54.5	—	—	40	2.4
Penicillium sp. 1 Link	20	1.0	20	2.6	—	—
Penicillium sp. 2	20	1.0	—	—	100	25.0
Penicillium sp. 3	20	1.0	—	—	80	13.1
Penicillium sp. 4	20	1.0	40	2.6	—	—
Penicillium sp. 5	—	—	20	5.1	—	—
Penicillium sp. 6	—	—	40	2.6	—	—
Penicillium sp. 7	—	—	—	—	20	1.2
Penicillium corylophilum Dierckx	—	—	—	—	40	2.4
Penicillium herquei Bainier and Sartory	—	—	20	1.3	60	7.1
Penicillium janthinellum Biourge	100	19.2	20	2.6	—	—
Penicillium rubrum Sopp	—	—	20	1.3	—	—
Ramichloridium sp. Stahel ex de Hoog	20	2.0	—	—	—	—
Rhizopus sp. Link	—	—	40	6.4	—	—
Rhizopus stolonifer (Ehrenb.) Vuill.	—	—	20	1.3	—	—
Trichoderma sp. 1 Pers.	—	—	20	2.6	—	—
Trichoderma sp. 2	—	—	20	2.6	20	1.2
Trichoderma sp. 3	—	—	—	—	40	2.4
Trichoderma polysporum (Link) Rifai	20	6.1	—	—	20	1.2
Trichoderma pseudokoningii Rifai	—	—	—	—	60	6.0
Verticillium lecanii (Zimm.) Viégas	—	—	20	1.3	—	—
Zygorhynchus moelleri Vuill.	20	2.0	20	7.7	20	1.2

Of the 100 fungal isolates from each location (fire ant mounds, nonmound soil, and *Aphaenogaster* nests), 1, 22, and 16% of the samples, respectively, could not be included in the analyses as a result of bacterial, yeast, or actinomycete growth. Fungal diversity was significantly ($F_{2,12} = 18.81$, df = 2, $P = 0.000$) lower in fire ant mounds, than in both *Aphaenogaster* nests and nonmound soil (Table 1). Species evenness was also significantly lower ($F_{2,12} = 23.81$, df = 2, $P = 0.000$) in fire ant mounds than in both *Aphaenogaster* nests and nonmound soil (Table 1). The number of fungal species in nonmound soil was higher than that found in fire ant mounds and *Aphaenogaster* nests (Table 1). Based on coefficients of similarity, there was little overlap in fungal species between sites. Fire ant mounds shared only 23.3% of fungi with adjacent, nonmound soil and 36.4% of fungi from *Aphaenogaster* nests. Nonmound soil and *Aphaenogaster* nests had the lowest coefficient of similarity (20.8). Out of 46 total fungi identified, only one, *Zygorhynchus moelleri* Vuill., was found in all three sites (fire ant mounds, nonmound soil, and *Aphaenogaster* nests); however, its relative colony frequencies were low (2.0, 1.2, and 7.7, respectively).

Discussion

Fire ant mounds contained 19 times more fungal colonies (cfu g^{-1}) than adjacent soil. Our results are consistent with those of Czerwiński et al. (1971) who found a 10-fold increase in the number of cfu in *Lasius niger* L. mounds and a three-fold increase in *Myrmica*

sp. Latreille mounds in comparison with uninhabited soil. Czerwiñski et al. (1971) did not identify fungal species, but we found that one species, *P. byssina*, dominated fire ant mounds. This fungus also was found in *Aphaenogaster* nest samples but in low relative numbers. Based on collection information, Hotson (1917) suggests that *P. byssina* is limited to horse dung; however, little is known about this fungus. Because *Aphaenogaster* spp. consume Agaricales mushrooms (Carroll et al. 1981), it is possible *S. invicta* and *A. texana carolinensis* are gathering *P. byssina* for food. However, we found that fewer fire ants foraged on cultures of *P. byssina* than on uninoculated agar. Our study was limited to one site, and more research is needed to determine if the ant-fungal associations we identified are widespread.

Fire ant mounds had lower fungal species richness, diversity, and evenness than *Aphaenogaster* nests (Table 1). Because *Aphaenogaster* nests average 12 cm deep, and upper nest chambers consist of leaf litter (Talbot 1951), these ants do not make discernable mounds. Due to the shallow construction of *Aphaenogaster* nests, less soil and more leaf litter was obtained from *Aphaenogaster* nest chambers than in *S. invicta* mounds. Therefore, lack of similarities between fungal species in fire ant mounds and *Aphaenogaster* nests might be explained by differences in nest construction.

We found that both fire ant mounds and native ant nests had lower fungal species richness and diversity than nonmound soil (Table 1). Differences among fungal diversity in *Aphaenogaster* nests, fire ant mounds, and the nonmound soil might also be a result of variations in environmental conditions such as temperature, moisture, nutrients, pH, and aeration in these microhabitats. Alternatively, these fungal differences might be due to the presence of antimicrobial defenses in ants and degrees of fungal resistance to these defenses. For example, secretions from metapleural glands can significantly reduce hyphal growth in *Aspergillus niger* Tiegh, *Cladosporium resinae* (Lindau) DeVries, *Gliocladium roseum* Bain, *Penicillium aurantiogriseum* Dierckx, *Trichoderma viride* Pers., *Beauveria bassiana* Bals. Vuill., and *Paecilomyces lilacinus* (Thom.) Samson (Beattie et al. 1985). Although metapleural secretions are nonspecific antimicrobials on gram-positive and gram-negative bacteria and yeasts (Veal et al. 1992), Beattie et al. (1985) found that one fungal species, *Metarhizium brunneum* Petch, showed resistance to metapleural secretions. Alkaloids from venom glands of *S. invicta* inhibit conidial germination, but they have little effect on hyphae (Storey et al. 1991). Therefore, it is possible that vegetative bulbils of *P. byssina* are resistant to fire ant defenses. In addition, fungi themselves can produce antibiotics and antifungal agents, which can inhibit growth of other fungal species. For example, *P. janthinellum* is known to produce agents that can limit the growth of other fungal species (Domsch et al. 1980). This may, in part, explain why nonmound soil contained higher fungal diversity than ant-occupied soils (Table 1). In addition, we found the highest proportion of bacteria, yeasts, and actinomycetes in our isolates from nonmound soil (22% contaminated), whereas fire ant mounds had the lowest percent (1% contaminated). We found that fire ant mounds had the highest cfu g^{-1}; however, species richness in fire ant mounds was reduced by over 50% compared with the surrounding soil. These high densities of limited numbers of fungal species in fire ant mounds possibly indicate that only some species (such as *P. byssina* and *P. janthinellum*) are tolerant to and thrive in mound conditions. Additional studies are needed to determine specific factors that limit fungal diversity in ant-occupied soils.

Native ants in the genus *Aphaenogaster* are involved in an apparent ant-seed mutualism whereby plants (myrmecochores) recruit ants to disperse seeds to their nests, which are favorable sites for seedling establishment (Handel 1978). Once ants discard the seed, both pathogenic and nonpathogenic fungi in the soil can hinder seedling recruitment by lowering seed viability (Baskin and Baskin 1998). It is unknown how differences in fungal populations in ant-occupied soil can affect the apparent mutualism between native, seed-dispersing ants and myrmecochorous plants. We found a reduction in fungal species in ant-occupied soils. Plants grown in ant nests might be more successful than those plants in surrounding soils (Culver and Beattie 1980) because seeds taken to ant nests might benefit by a reduction in plant pathogens. Ant nests might even provide ideal growing conditions for slowly developed mycorrhizae associated with myrmecochores (Brundrett and Kendrick 1990). Further studies are needed to investigate the fungi found in ant-occupied soil to explain any possible advantage of having directed seed dispersal.

The introduction of non-native ants into fynbos habitats in South Africa has negatively affected myrmecochores by reducing seedling emergence, and Bond and Slingsby (1984) suggested that this introduction can impact plant community composition by reducing seedling recruitment. In the southeastern United States, fire ants are attracted to and destroy the seeds of myrmecochores, and seeds that escape consumption have their seed coat removed (scarified) (Zettler et al. 2001). Scarification of seeds can allow fungi to invade and cause seed mortality (Kremer and Spencer 1989). Through the production of enzymes and toxins, fungi can cause seed mortality (Baskin and Baskin 1998). Once fire ants abandon a mound, it is unknown how long fungal differences between the nonmound and mound soil will remain. Through frequent colony movement, fire ants might have long-term effects on soil fungi. Thus, differences in fungi found in fire ant mounds and native ant nests may further disrupt the mutualistic relationship between native ants and myrmecochores.

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