THE DIRECT AND INDIRECT EFFECTS OF THE RED IMPORTED FIRE ANT (*SOLENOPSIS INVICTA*) ON SEED FATE IN THE LONGLEAF PINE ECOSYSTEM

by

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(Under the direction of L. Katherine Kirkman)

ABSTRACT

Fire ants have invaded and become established throughout the southeastern United States, primarily in environments with highly disturbed vegetation. We examined the influence of timber harvest disturbance on ant community composition in natural longleaf pine stands with fire ants present. We also examined the direct impacts of fire ants on seed removal and seed fate. We found no change in the ant community in response to harvest treatments applied in this study, but effects may appear over time. Fire ants were the dominant seed remover in this area, and preferentially removed seeds with elaiosomes. Once elaiosome-bearing seeds were removed by fire ants, a third were found deposited in trash piles on the soil surface. No seeds were located in nest chambers. We found no evidence that ants caused a decrease in ant-manipulated seed germination. Our findings indicate that fire ants do facilitate some movement of seeds in the longleaf ecosystem.

INDEX WORDS: Fire ant, *Solenopsis invicta*, Myrmecochory, Seed dispersal, Elaiosome, Invasive ant, Disturbance, Native ants, Ecosystem resilience, Longleaf pine, Ecological forestry

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Project Overview

Ants play a significant part in many ecosystem processes. Native ant species are important predators, herbivores, granivores, mutualists (Hölldobler and Wilson 1990), and create habitat (Folgarait 1998). Because many ant species are highly adaptable to a wide range of environmental conditions, numerous ant species have been successfully introduced into new environments, where they have had significant impacts or even become major pests (Holway et al. 2002). Invasive ant species usually become established in areas that are highly disturbed, particularly anthropogenic disturbances that disrupt soil and vegetation (King and Tschinkel 2008). Invasive ant species can affect native vegetation by altering a mutualistic relationship between native plant and native ant species centered on the dispersal of seeds (Christian 2001, Gómez and Oliveras 2003). Ant-dispersed (myrmecochorous) plants often exhibit slower rates of dispersal due to shorter distances of movement than other forms of dispersal (Matlack 1994, Cain et al. 1998). Hindrances to dispersal by invasive species may further slow or even eliminate re-colonization (Kirkman et al. 2004). One particularly infamous invasive ant species is the red imported fire ant *Solenopsis invicta,* noted for its rapid expansion in the 20th century and occurrence throughout disturbed areas of the southeastern United States (Callcott and Collins 1996). Their overall effect on native ant species, as well as myrmecochorous plant species, is not well known (Holway et al. 2002, Stuble et al. 2010). This study examines how different timber harvest techniques that result in a gradient of soil and canopy disturbance can alter an ant community composition that initially includes an established invasive ant species. The research also examines how the red imported fire ant, by collecting seeds, affects myrmecochorous seed dispersal.

The following literature review summarizes the importance of myrmecochory and the potential impacts of an invasive ant species on this mutualism, as well as the distribution of the red imported fire ant relative to disturbance environments. The final section outlines the research objectives and contents of the remaining three chapters. Hereafter I will use the term "fire ant" to refer to only the red imported fire ant, *Solenopsis invicta*.

Literature Review

Myrmecochory and Invasive Ants

Myrmecochory is an important mutualistic relationship between ants and plants (Hölldobler and Wilson 1990). Myrmecochorous plants evolved independently in at least 77 plant families (Lengyel et al. 2009), but most have a fatty appendage, called an elaiosome, attached to their seeds (Sernander 1906). Ants transport elaiosome-bearing seeds to their nest or under leaf litter, remove the elaiosome as a food resource, and then discard the seeds into trash piles within or near the nest. Ants benefit from the elaiosome, which contains lipids beneficial to their larvae (Beattie 1985), and seed survival is increased in at least one of several ways. The seeds may gain an advantage from being deposited in underground trash piles through protection from above ground seed predators and/or fire (Berg 1975, Reichman 1979); such microsites may contain higher nutrient levels than the surrounding soil due to decaying ant corpses and extra food bits (Beattie 1985). Also, seed removal away from the mother plant is thought to increase survival probability (Andersen 1988).

Omnivorous and carnivorous ants are most likely the main distributors of myrmecochorous seeds, given that elaiosomes contain chemicals similar to those found in insect hemolymph (Hughes and Westoby 1992, Rico-Gray and Oliveira 2007, Servigne and Detrain 2008). Granivorous ants will also collect elaiosome-bearing seeds, but the end effect is primarily predatory. In such cases, only those few seeds lost during transport would be able to benefit (Rico-Gray and Oliveira 2007).

Myrmecochory has evolved in at least 334 genera found in 77 families of angiosperm species worldwide (Lengyel et al. 2009). These species are particularly abundant in two fire-adapted communities, such as the fynbos in Africa (Bond and Slingsby 1984) and the shrub-dominated regions of Australia (Berg 1975), as well as the herbaceous understory in north temperate mesic forests (Beattie and Culver 1981, Handel et al. 1981, Lengyel et al. 2009). The ant genera involved in this mutualism vary in life history traits and the ant-plant relationship is not species specific, which allows myrmecochory to be affected by the presence of non-native ant species.

Invasive ants can affect myrmecochory directly by collecting seeds and depleting the seed bank (Holway et al. 2002, Ness and Bronstein 2004) or by dispersing seeds; indirectly, they may affect native ant populations that are seed dispersers. For example, the major invasive ant species in the fynbos, Argentine ant *Linepithema humile,* does not collect seeds. Instead, the Argentine ant disrupts dispersal through competitive displacement of seed-dispersing native ants (Bond and Slingsby 1984, Christian 2001, Carney et al. 2003, Gómez and Oliveras 2003).

Impacts of Fire Ants

Invasive ants are usually characterized by their wide environmental tolerance, high number of workers, and aggressive behavior (Holway et al. 2002, Buczkowski and Bennett 2008). Fire ants embody all these characteristics. Originating in South America, they have extensively expanded into the United States and from there to the eastern hemisphere, invading New Zealand, Australia, and Eastern Asia (Xiong et al. 2008). Strong venom and high recruitment numbers, coupled with their tendency to take advantage of human-disturbed areas, have made fire ants one of the most successful, and most hated, invasive species. Fire ants were introduced at the port in Mobile, Alabama in the 1930s, perhaps in soil used as ballast or packing material (Callcott and Collins 1996, Vinson 1997). Currently their range in the United States reaches from east Texas to eastern Virginia with isolated spots in California, but their projected range in the US is much greater (Fitzpatrick et al. 2007).

The primary mechanism found to control fire ant territory size in homogenous habitats, such as pastures, is the territories of other fire ant colonies. This mechanism is only present in monogyne (single queen) colonies (Adams and Tschinkel 2001). Polygyne (multiple queens) colonies can reach even higher densities due to lack of intraspecific competition (Vinson 1997). The main control on fire ants in the native range is competition from other aggressive ant species (Porter et al. 1997, Calcaterra et al. 2008). Its explosive populations in the United States are attributed to a release from this competition.

Fire ants injure or kill native wildlife, especially young birds, reptiles, and mammals (Allen et al. 1994, Wojcik et al. 2001, Wetterer and Moore 2005, Diffie et al. 2010). They also depend on sugary food and sometimes damage plants by collecting sap directly or through aphid tending, thus decreasing plant fitness (Smittle et al. 1983 and 1988, Kaakeh and Dutcher 1992).

Fire ant venom causes human health problems by occasionally producing severe allergic reactions in 1% of the population (Lofgren et al. 1975). In agricultural areas, fire ants attack livestock, damage farm equipment, and sometimes even negatively affect crops (Lofgren et al. 1975). The economic cost of damages by and control of fire ants in the southern United States is more than $1 billion per year (Pimentel et al. 2000). Excavations by fire ants in nest building alter soil structure (Green et al. 1999) and soil nutrients (Lafleur et al. 2005). Whether this interaction is positive or negative depends on the benefits of mixing soil layers by soil type. These effects by fire ants are summarized in Figure 1.1.

Many arthropod populations decrease immediately following a fire ant invasion, including species of native ants (Porter et al. 1988, Hook and Porter 1990, Porter and Savignano 1990, Morris and Steigman 1993, Wojcik et al. 2001). However, in one follow-up study 12 years after the fire ant invasion, species richness and abundance of native ants had returned to pre-invasion levels (Morrison 2002). Although most pre-invasion data on ant community species richness is lacking, recent studies suggest that ant species richness is independent of fire ant density (Graham et al. 2004, MacGown and Brown 2006, King and Tschinkel 2008, Stuble et al. 2009). In some cases, native ant species abundances were shown to be inversely proportional to fire ant density, suggesting some interspecific exclusion of fire ants by native ants (Rao and Vinson 2004, Tschinkel 2006, Stuble et al. 2009).

Fire Ants and Disturbance

Views about the role of disturbance on fire ant establishment have evolved over the last several decades. Initially, observations of fire ant invasion were primarily associated with highly disturbed areas, such as urban and agricultural settings (Wilson and Oliver 1969, Lofgren et al.

1975, Tschinkel 1988). Fire ants were thought to be absent in forest interiors (Tschinkel 1988, Zettler et al. 2001), though they were occasionally found in high numbers within some natural areas, specifically secondary hardwoods surrounded by grassy fields (Hook and Porter 1990, Porter and Savignano 1990). There, fire ants were viewed as destructive invaders that decreased native ant populations and reduced species richness. King and Tschinkel (2008) refer to fire ants as restricted to disturbed areas and absent from undisturbed pine flatwoods in northern Florida. However, fire ants are found within naturally regenerated second growth longleaf pine forests with native ground cover, as well as old growth longleaf pine forests in southwest Georgia (Carroll and Hoffman 1997, Lubertazzi and Tschinkel 2003, Graham et al. 2004, Tschinkel 2006, Stuble et al. 2009 and 2010, Carroll *personal communication*).

Few stands of longleaf pine forests, especially those with a native ground cover undisturbed by agriculture, remain in the southeastern United States. Even most remnant tracts of fire-maintained longleaf pine forests are riddled with hardwood forests, roads, and fire breaks. In addition, ecological forestry practices that include timber extraction introduce disturbances, regardless of attempts to protect ground cover and minimize soil disturbance (Franklin et al. 2002). The basic premise of ecological forestry involves finding a balance between health and profitability of an ecosystem, which is often discovered by measuring ecosystem resilience. One measure of resilience that has been applied to several forest ecosystems following disturbance has been that of the ant community composition (Andersen 1991, Roth et al. 1994, Majer 1996, Oliver et al. 1999, Andersen et al. 2002, Andersen and Majer 2004, Graham et al. 2004, Andersen et al. 2009, Arnan et al. 2009, Lomov et al. 2009, Dominguez-Haydar and Armbrecht 2011). Consequently, the role of disturbance and fragmentation on the vulnerability of a longleaf pine forest to fire ant invasion and proliferation, and subsequent impacts on native ant populations, is of interest in assessing ecological forestry impacts.

Studying the impacts of fire ants on native plant communities is often impeded by other factors that also influence vegetation. Fire ants preferentially invade open areas with soil disturbance, especially pastures, cultivated areas, roads, or urban development sites (Stiles and Jones 1998, Zettler et al. 2004, King and Tschinkel 2008). Presumably, the lack of soil disturbance and closed canopy conditions have prevented invasion of fire ants into most native forests. Thus, most studies of the interaction between fire ants and plant communities have been conducted in artificial settings or highly disturbed environments (Ready and Vinson 1995, Zettler et al. 2001, Seaman and Marino 2003). The invasion of fire ants into the native longleaf pine ecosystem provides an opportunity to observe how fire ants affect a naturally occurring plant community that has been relatively undisturbed by human manipulation of soil or vegetation. The longleaf pine ecosystem is one of the most floristically diverse in North America (Kirkman et al. 2001). Several common plant species are known to be myrmecochorous, though the percentage is less than one percent of species overall due to the high diversity of plant species (Stamp and Lucas 1990, Kirkman et al. 2001, Stuble et al. 2009).

Fire Ants and Seed Dispersal

Fire ants are reported to carry seeds into foraging tunnels (Wilson and Oliver 1969, Tennant and Porter 1991, Stuble et al. 2009, *personal observation*) and remove and consume the elaiosome of myrmecochorous seeds (Zettler et al. 2001). They are even reported to consume or destroy the seed itself, including several non elaiosome-bearing species (Ready and Vinson 1995, Zettler et al. 2001, Seaman and Marino 2003). However, conclusions as to the degree to which this interaction positively or negatively affects dispersal success of elaiosome-bearing seeds in a natural environment are inconsistent. For example, Ness (2004) found the seed dispersal distance by fire ants was significantly shorter than that of native ants. In contrast, Stuble et al. (2009) concluded that fire ants move seeds distances equivalent to that of native ant species. This discrepancy may be due to the amount, species composition, and landscape context of the ground cover affecting travel by ants during these observations. Ness's study was conducted on a mesic deciduous forest edge characterized by dense leaf litter, while Stuble's study was conducted in a frequently burned wiregrass-dominated longleaf pine forest, presumably with less hindrance to movement along the ground surface.

In longleaf pine ecosystems, fire ants were observed to be the dominant seed-removing ant species (Carroll and Hoffman 1997, Stuble et al. 2009). Foraging tunnels that radiate out around the mound are 2-10 cm below ground. Once a seed is taken underground into a foraging tunnel, its fate is difficult to determine (Markin et al. 1975, Tschinkel 2006). Studies by Wilson and Oliver (1969) and Tennant and Porter (1991) found that seeds comprised 0.5% to 4.8% of the solid foods brought into the nest, but no identification of seeds was attempted in either study. Seed collection by fire ants may depend on the presence of seeds with elaiosomes (Garrido et al. 2002), or the overall seasonal abundance of food. Fire ant preference would determine which seeds are most affected by an altered dispersal pattern. Studies examining seed preference by fire ants found they readily collect and consume some seeds, both with and without elaiosomes (Ready and Vinson 1995, Zettler et al. 2001), but no experiments have observed consumption or preference of seed by fire ants within a natural plant community. Arthropods make up a large percentage of solid food collected by fire ants (Wilson and Oliver 1969, Tennant and Porter 1991). Therefore, a fire ant's preference for elaiosome-bearing seeds would, perhaps, be due to their attraction to the elaiosome (Hughes et al. 1994, Rico-Gray and Oliveira 2007, Servigne and Detrain 2008). This attraction to certain seed species over others may impact overall seed dispersal in ecosystems invaded by fire ants.

Fire ants have displaced two closely related, native species of fire ant in much of its range, the southern fire ant (*Solenopsis xyloni*) and the native (or tropical) fire ant (*Solenopsis geminata*) (Vinson 1997). *S. geminata* is a seed predator, sometimes even considered a granivore (Hölldobler and Wilson 1990) while *S. xyloni* is usually considered an omnivore (Trager 1991) even though this species collects and stores seeds in the summer (Valone and Kaspari 2005). Seed predation by the native fire ant colonies were likely greater than that of an invasive fire ant colony. However, the greater number of foragers, as well as colonies, of the invasive fire ant may lead to greater seed removal events overall (Tennant and Porter 1991). Even though the native fire ant's impact as a seed disperser is not known, it likely differed from that of the invasive fire ant. Since fire ants are the dominant seed removers in most of their invaded range, the fate of seeds displaced by fire ants has potential implications for dispersal and recruitment of plant species, particularly in the restoration of native ground cover in the longleaf pine ecosystem.

The purpose of this research is to examine the effects of disturbance on an ant community with an established fire ant population, and determine the effect of fire ants on seed fate in a longleaf pine ecosystem. If the invasive fire ant prevents seeds from germinating, they would hinder the re-colonization of native myrmecochorous species. However, if fire ants are providing some level of seed dispersal, controlling their population in restoration areas would be less vital to success. Our examination of management options for timber harvest will also assist in restoration by determining the effect of harvest techniques on the resilience and recovery of the longleaf pine ecosystem by measuring the ant community as an indicator of disturbance.

Research Objectives and Thesis Format

The overall objective of this study is to determine how fire ants affect seed dispersal in the longleaf pine ecosystem. Specifically, this thesis quantifies the change in the ant community and impacts on seed removal due to disturbance (Chapter 2), and investigates the fate of seeds collected by fire ants (Chapter 3). Chapter 4 summarizes our findings and their importance. The research addresses the following questions:

Chapter 2

1. How does canopy and soil disturbance associated with timber extraction within a natural longleaf pine stand affect ant community one year post-harvest?
2. Does disturbance impact the rate of seed removal by ants?
3. Is past disturbance history a predictor of the ant community?

Chapter 3

1. Which plant species or types of seed are preferentially removed by fire ants?
2. Are seeds removed by fire ants deposited above ground (into trash piles) or below ground (into nests)?
3. Does ant manipulation alter germination success of elaiosome-bearing seed?

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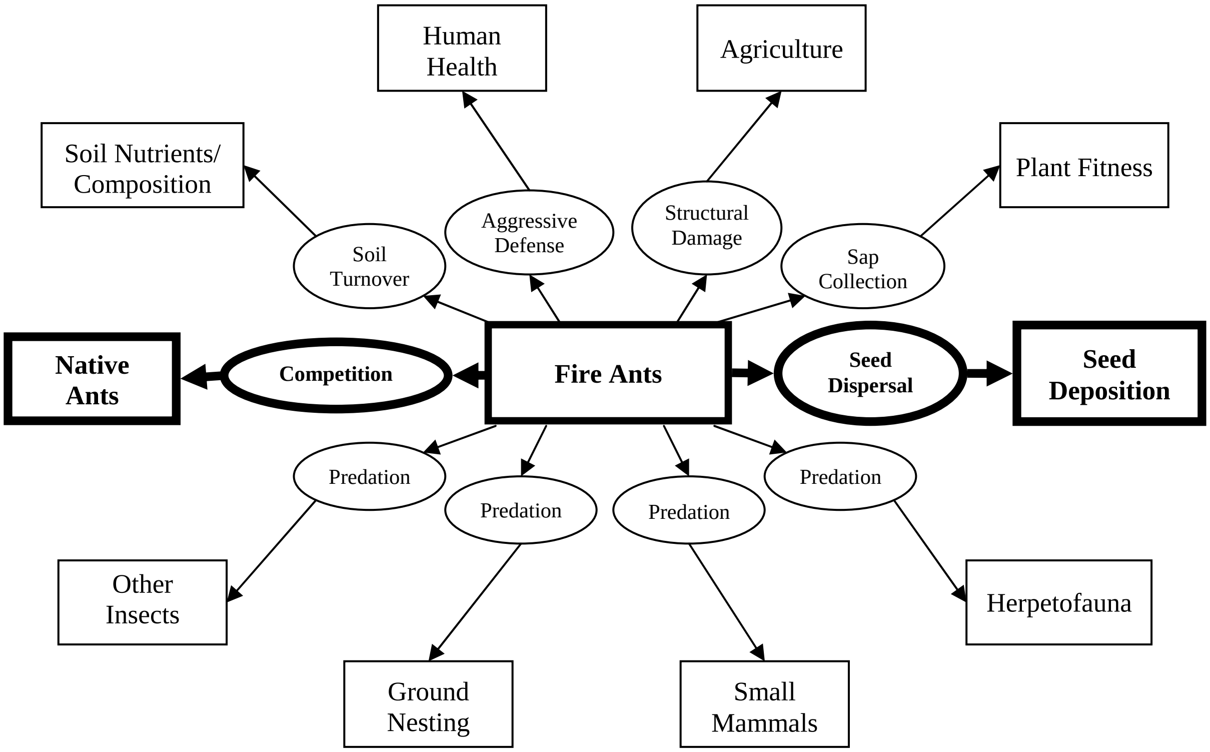
Figure 1.1 Fire ant impacts on biological and societal properties. Circles indicate an action by fire ants on the subject in the boxes. Bolder outlines indicate areas covered in this study.



CHAPTER 2

THE EFFECTS OF DISTURBANCE ON THE RED IMPORTED FIRE ANT AND THE NATIVE ANT COMMUNITY[1]

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[1] M.E. Shearin. To be submitted to *Journal of Forest Ecology and Management*.

Abstract

The red imported fire ant (*Solenopsis invicta*) is one of the most successful, and most hated, invasive species in the southeastern United States. Fire ant numbers tend to be elevated in highly disturbed environments, such as roadsides, cultivated fields, and lawns. Although fire ants have invaded and even dominate some native communities in the southeast, for instance the longleaf pine, their response to disturbances such as timber harvests in these environments is unknown. Fire ant presence and dominance, coupled with immediate or even past disturbance, may have an effect on the native ant community. We used four timber harvest treatments (control, group selection, group selection with retention, and single tree selection) to measure the effect of disturbance on the fire ant and native ant community. We used seed removal rates to determine if behavior was also affected. We collected ants using pitfall traps located in 18 treatment plots, with 20 collections per plot. Seed removal took place in 4 of the treatment plots with 19 observations before and 19 after the timber harvest in each treatment. We also compared the ant community between areas of historically plowed and unplowed soil using the dominate ground cover as an indication of past disturbance.

Fire ants were by far the most abundant ant and the most frequent seed remover in this longleaf pine ecosystem, regardless of silvicultural treatment. The disturbances due to timber harvest did not result in an initial change in abundance of fire ants or native ants, total species richness, or rate of seed removal. Habitat changes such as reduced canopy cover and increased woody debris may influence changes in the ant community over time. Pre-treatment differences in ant community assemblage between blocks suggest that past disturbance may have more of an effect on the distribution of ant species, but this observation is not associated with historical soil disturbance indicated by wiregrass presence. One experimental block studied was unique prior

to harvest treatments in that the fire ant population was extremely low. The cause of this absence is unknown and needs further examination.

Introduction

Knowledge of ecosystem response to natural and anthropogenic disturbances can provide useful information in developing resource management techniques and prescriptions. Disturbances, such as frequent fire or canopy gaps, are often crucial ingredients necessary to maintain native species, high biodiversity, or promote uneven-aged stands of trees (Archer 1988, Leach and Givnish 1996, Grassi et al. 2004). Inevitably, economic extraction of resources, such as timber, involves disturbances that will affect ecological processes (Alexander et al. 2002). In some cases, important natural disturbances may be mimicked by the silvicultural practice to encourage ecosystem resilience (Brown et al. 1991, Gundale et al. 2005, Hutchinson et al. 2005). Consequently, the basic premise of ecological forestry requires finding the balance between sustainability of ecosystem health and profitability (Bradstock et al. 1998, Franklin et al. 2002).

Invasive species tend to take advantage of disturbance because of the disruption of community competitive relationships and increased availability of space and nutrient resources (Alpert et al. 2000, Holway et al. 2002, Taylor and Irwin 2004, MacDougall and Turkington 2005, Leprieur et al. 2008). Native species are then affected by both disturbance and the subsequent increase in invasive species (Williams 1994, Fowler 1995).

The composition of native ant communities has been identified as a useful indicator of disturbance and the success of restoration efforts in many forest ecosystems worldwide (Andersen 1991, Roth et al. 1994, Majer 1996, Oliver et al. 1999, Andersen et al. 2002, Andersen and Majer 2004, Graham et al. 2004, Andersen et al. 2009, Arnan et al. 2009, Lomov et al. 2009, Dominguez-Haydar and Armbrecht 2011), but how native ant colonies will react to disturbance when their area is dominated by an invasive ant species is unknown. The response between the timber harvest and ant community needs to be clarified to determine the effect the ant community will have on native seed dispersal, especially within logged areas.

The red imported fire ant (*Solenopsis invicta*) is a major invasive species that cause many problems to wildlife, agriculture, and human health worldwide (Allen et al. 1994, Wojcik et al. 2001, Wetterer and Moore 2005, Diffie et al. 2010). Fire ants tend to invade open and disturbed areas, particularly soil disturbances introduced by human actions (Tschinkel 1988, DeHeer et al. 1999, King and Tschinkel 2008). The effects of a fire ant invasion on native ants are not well-defined, but in many cases it has been found that after an initial displacement of native species, fire ants subsequently coexist with a high diversity of native ant species (Morrison 2002, MacGown and Brown 2006, King and Tschinkel 2008).

Our study of ant community dynamics is part of a collaborative long-term project to examine the ecosystem impacts of disturbances associated with various silvicultural approaches in longleaf pine forests. The overall objectives of the long term study are to quantify the role of canopy retention on fine fuels and fire behavior, and the resilience of the ground cover diversity, with regard to varying intensities of soil and canopy disturbance. In this study, we examined the initial response of the ant community due to disturbance. Because historical disturbances often create legacies that persist in the wildlife and plant community (Myers and Van Lear 1998, Franklin et al. 2002) we are also considering the effect of past disturbance on ground cover that may have influenced ant community composition prior to this study. Within the longleaf pine stands, the presence of a species-rich native ground cover and wiregrass (*Aristida stricta*) dominance are indicative of past intensive management disturbances such as bedding, scraping, and soil scarification (Myers 1990). Comparisons of these relatively undisturbed areas to natural stands of longleaf pines lacking a wiregrass-dominated ground cover may reveal differing patterns in the ant assemblages. This may reflect differences in species colonization history, or effects of the ground cover itself.

The purpose of this study is to quantify the change in an ant community in which the red imported fire ant is present and to examine the impacts on seed removal by ants in response to different levels of disturbance associated with timber harvest techniques. Specifically, we address the following questions: 1) How does canopy and soil disturbance associated with timber extraction within a natural longleaf pine stand affect ant community one year post-harvest? 2) Does disturbance impact the rate of seed removal by ants? 3) Is past disturbance history a predictor of the ant community?

Materials and Methods

Study Site

The study was conducted at the Joseph W. Jones Ecological Research Center at Ichauway, located in Southwest Georgia (Baker County). Ichauway maintains approximately 12,000 hectares of land dominated by second-growth longleaf pine (*Pinus palustris*) forest (approximately 90 years old), scattered agricultural fields, isolated depressional wetlands, and hardwood riparian corridors. Historically, this property was managed for northern bobwhite quail by frequent prescribed fire for many decades. Currently, prescribed burns are carried out at two-year return intervals. Over 30 native ant species have been documented in the longleaf pine forests at Ichauway (Carroll and Hoffman 1997, Stuble et al. 2009). Although the date of arrival by fire ants is not documented, they were present in the county by the mid-1960s (Callcott and

Collins 1996). The average daily temperature is 11ºC during winter and 27ºC during summer with an average annual rainfall of 137 cm per year (Georgia Automated Environmental Monitoring Network, University of Georgia).

Long Term Ecological Forestry Project

The overall experimental design of the long term silvicultural study is an incomplete randomized block design. A complete block design was not possible owing to availability of forest stands for harvest configuration and the number of treatments. Each plot was 4 hectares, and there were 18 plots total. Half of the plots were established on historically unplowed areas with intact wiregrass-dominated herbaceous ground cover. The other nine were established on historically plowed areas consisting of naturally regenerated longleaf forest dominated by forbs and grasses other than wiregrass (hereafter referred to as old field). Six blocks, containing three plots each, were established based on location, similar tree basal area, and ground cover condition (Figure 2.1). Each block contained one plot with single trees selected for harvest based on the Stoddard-Neel approach (Jack et al. 2006). The remaining two plots in each block were each randomly assigned to one of three remaining treatments: a) small to large group selection, b) small to large group selection with retention of canopy trees within gaps, or c) un-harvested control stands. Basal area of both group selection treatments matched the basal area of removed single trees on a per block basis. Plots within blocks were separated by at least 50 meters. The timber harvests were completed in November 2009. All blocks were burned during winter 2008 prior to pre-harvest ant sampling, and again in spring 2010 after harvest before the post-treatment sampling.

Ant Community (Pitfall Traps)

We used pitfall traps to monitor ground foraging ant species before and after treatments because biannually prescribed fire reduces litter build-up to levels too low for ant collection by litter samples. The traps were constructed as described by Majer (1978). Each trap consisted of a polyvinyl chloride pipe segment (15.3 cm long, 2.1 cm diameter), inserted into the ground with a rimmed test tube resting inside (150 mm long, 20 mm in diameter). The killing agent used was non-scented soapy water in the bottom of each test tube. While not in use, we closed each trap using a rubber stopper. In each of the 18 plots we established 20 pitfall trap groups to monitor disturbance effects on the ant community. Each group consisted of 4 pitfall traps arranged along a line one meter apart (Figure 2.2). This arrangement with a large edge area for ants to encounter was used to maximize the number of species caught per location (Abensperg-Traun and Steven 1995). The contents from each group of 4 pitfall traps were pooled into one vial, with 20 vials collected per plot (Figure 2.2). We chose the southwest corner of each quarter of a plot as a point of origin and plotted random coordinates in increments of 10 meters. Pitfall traps were removed from plots prior to timber harvest, and replaced in the same locations following harvest and the subsequent prescribed burn. Ants were sampled each month for four months before and four months after the timber harvest in June, July, August, and September. Pitfall traps were opened for approximately 23 to 27 hours in each sampling effort. Each effort sampled 9 plots which included 3 complete blocks. The pitfall traps were collected in two sampling efforts each month due to the large amount of area to travel. Sampling did not occur if rain was predicted within the trapping period.

All ants collected were sorted and identified to genus, and then to species when possible. The abundance of each ant species was also recorded. The term “native ant” refers to all ants that are not the invasive fire ant. This grouping does include two introduced ant species which either occur in very low numbers (*Cardiocondyla wroughtonii*), or is not known to cause any detrimental environmental effects (*Cyphromyrmex rimosus*) (Deyrup et al. 2000). Three taxa were only identified to genus due to difficulty of positive identification to species: *Phiedole* sp. similar to *P.dentigula*, *Paratrechina* sp. similar to *P. arenivaga*, and *Pyramica* sp. similar to *P. creightoni*. All specimens were stored in 90% ethanol are held at the Georgia Museum of Natural History in the University of Georgia, Athens, Georgia.

Seed Removal

To quantify the removal rate of elaiosome-bearing seed by ant species, we observed seed removal in a subset of treatment plots. We selected four plots (one of each treatment) that were in close proximity spatially and dominated with wiregrass ground cover (plots 13, 14, 15, and 18) (Figure 2.1). We completed nineteen observations before and nineteen observations after the harvest between the months of May and November, with three to four observations per month. For each observation, a Petri dish with four holes in the side for easy access by ants was placed in a randomly generated location within the plot. If the location of the Petri dish fell on a visible fire ant mound, the observation was shifted 1 m away to eliminate bias.

Ten seeds of *Piriqueta cistoides,* a common elaiosome-bearing species readily collected by ants (Chapter 3), were placed in the middle of the dish and observed until ants collected all 10 seeds or one hour had passed, whichever came first. We recorded the ant species that collected each seed. A seed was considered removed if the ant carried it completely off of the dish. Observations were conducted in the morning, and all four plots were visited in a randomly established order.

Data Analysis

Pitfall trap data was first sorted to eliminate outliers (extremely high numbers of one species in a single vial). These outlier abundances usually occurred when an ant species recruited with a large number of individuals to a single pitfall trap, either to consume other trapped insects, or rarely, to consume water. Our criteria to identify outlier data was, when one species in a vial exceeded 100 individuals more than the median for all 20 vials of that species within a plot. The mean number of ants, excluding the outliers, was then calculated in each plot for total number of ants, and for both native ants and fire ants separately. To test for differences among treatments for mean native ant abundance per vial, fire ant abundance per vial, total ant abundance per vial, and relative abundance of fire ants and native ants, we used analysis of covariance (ANCOVA) with the pre-harvest data as the covariate (PROC GLM, SAS version 9.2). The ANCOVA uses the covariate to control for differences between plots that persist from year to year.

We also repeated the analysis independent of harvest treatment with plots classified by three different categories of soil disturbance (Table 2.1): 1) the total area of the plot that was disturbed by skid trails, 2) the total number of skid trails and skid trail passes within each plot, and 3) the number of individual trap sites within a skid trail or tire track per plot. To compare differences between means due to disturbance levels, we used the Tukey's Honestly Significant Difference multiple comparison test (SAS version 9.2), with significance level 0.05.

We determined the probability of five native ant species occurring in the same locations as fire ants with a logistic regression (PROC LOGISTIC, SAS version 9.2). We tested this relationship with the most abundant native ant species (*Phiedole sp*., *Brachymyrmex depilis*, *Solenopsis carolinensis*, *Dorymyrmex bureni*, and *Phiedole dentata*).

We generated species richness curves and species richness estimates from pitfall trap data with EstimateS version 8.2 (Colwell 2009). This analysis is useful for determining if sampling was adequate, and for estimating the true species richness (Gotelli and Colwell 2001). We used three estimators for estimating species richness (ICE, Chao 2, and Michaelis Menten) that have proven useful for estimating ant species richness (Longino 2000). The Michaelis Menten (M-M) richness estimator is a commonly used parametric measure, while ICE and Chao 2 are nonparametric measures based on incidence data, thus controlling for abundance differences due to recruitment (Colwell and Coddington 1994). Based on equal sampling effort, the final estimates of species richness and the observed species richness were also considered the species density within the plots. The mean species richness and estimates were compared between harvest treatments and soil disturbance classes using an ANCOVA with the associated before harvest values as the covariate (PROC GLM, SAS version 9.2).

To examine change in ant community composition with abundance and species distributions by plot we used Nonmetric Multidimensional Scaling (NMS). The mean ant abundance per species without outliers was used as the response variable for each plot. The NMS analyses were conducted in R (R Development Core Team 2010) using the *labdsv* package (Roberts 2010) and the *vegan* package (Oksanen 2010). The initial NMS analyses were run on a community matrix of 36 plots (both years) and 25 species using the Sørensen distance measure, a procedure in which the solution was stepped down from six to one-dimensions as recommended in McCune and Grace (2002). Stress, a measure of the poorness of fit between the original ecological distance and the ordination distance (McCune and Grace 2002), was plotted against the number of dimensions in a scree plot to determine the appropriate number of dimensions. The final NMS solution had two dimensions and a low stress value of 9.634. Post NMS processing was conducted to ensure the origin was positioned at the average of the axes, and principal components were used to rotate the final configuration to maximize variance on the first axis. A Shepard plot in which the original ecological distances were plotted against the ordination distances showed that the ordination effectively fit the community data ($R^2 = 0.97$). We examined the change in ant community composition with the direction of the vectors between pre- and post-harvest and comparing the degree of angle from axis 1.

To test for significant pair-wise and across-group differences in ant species composition by timber harvest treatment and soil disturbance classes after harvest, we used a rank-transformed Multi-response Permutation Procedure (MRPP) (Mielke 1984) conducted in PC-ORD version 5 (McCune and Mefford, 2006). Within-year analyses were conducted on a community matrix of 18 plots (one year) and 25 species using the Sørensen distance measure, while between-year analyses used the NMS scores and Euclidean distance to account for correlation among repeated measures samples.

We compared the mean seed removal rates between treatments before and after harvest (PROC GLM, SAS version 9.2), as well as comparisons each year for all ant species combined, with analyses of variance (ANOVA, PROC GLM, SAS version 9.2). To determine if seed removal varied between years, overall seed removal data was compared between before and after harvest using a t-test (PROC TTEST, SAS version 9.2). The pitfall trap data for these four plots was also analyzed to see if the mean abundance or relative abundance had changed to assist in explaining seed removal results. For this analysis, we used ANCOVA with before harvest mean abundance as the covariate (PROC GLM, SAS version 9.2).

Past Disturbance

To examine the possibility of historical land-use legacies affecting pre-harvest ant composition, we examined differences in mean and relative abundance of fire ants and native ants prior to harvest due to blocks based on location with an ANOVA (PROC GLM, SAS version 9.2) and the wiregrass presence with a t-test (PROC TTEST, SAS version 9.2). We also conducted the same analyses for mean species richness. Finally, we used MRPP to examine the potential impact of past disturbance on ant species composition using the presence of wiregrass as an indicator of no previous intensive soil or ground cover disturbance (R Development Core Team 2010).

Results

Ant Community (Pitfall Traps)

Overall, we collected 16,154 individual ants in 2009 and 16,681 individuals in 2010 belonging to at least 25 species within 19 genera. By far, the fire ant was the most abundant and most frequently sampled ant, occurring in 62% of all vials. The second most abundant ant, *Pheidole* sp., occurred in only 34% of all vials. Total number of individuals decreased over the months of sampling each year in accordance with the seasonal change (Figure 2.3). There are no differences in mean abundance or relative abundance of fire ants or native ants between any of the treatments or soil disturbance classes ($p > 0.05$) (Table 2.2).

Of the ant species examined, *B. depilis* had the highest probability of occurring with fire ants (0.783), with *D. bureni* closely following (0.749). Although *Pheidole* sp. (0.538) and *S. carolinensis* (0.516) had a much lower probability of occurring with fire ants, they were found more often with them than otherwise. Only one ant species, *P. dentata,* (0.444) was slightly more often found in areas without fire ants (Table 2.3).

Based on asymptotic estimates of species richness (Colwell and Coddington 1994), the amount of sampling was adequate for all dates except August 2010 (Figure 2.4). Observed mean species richness did not differ as a result of experimental treatments (df = 3, f = 2.59, $p > 0.05$) or soil disturbance classes (Area of disturbance f = 0.44, $p > 0.05$; Number of skid trail passes f = 2.64, $p > 0.05$; Individual pitfall trap disturbance f = 0.66, $p > 0.05$). Of the estimates of species richness, Chao 2 did not reveal any differences in species richness, but comparisons between treatment levels with the other two estimates resulted in some differences. The M-M richness estimates were significantly different between treatments (df = 3, f = 3.54, $p < 0.05$). The group treatment and the group with retention treatment had significantly higher mean species richness than single tree selection, but group with retention was the only treatment significantly higher than the control. The ICE species richness estimates were significantly different between the number of skid trail passes (df = 3, f = 3.00, $p < 0.05$) where a medium level of skid trail passes was significantly higher in estimated species richness than areas with fewer skid trail passes. Both of these differences, though significant, were not mirrored in the observed species richness, or other estimates of species richness. When the trends of species richness estimators do not agree, differences are more likely to be due to computation of estimates rather than applied treatments.

Based on the MRPP analyses of the after harvest data (18 plots), there were no differences in ant community composition between harvest treatments. There was a difference in ant community composition between the low area of disturbance level and all other disturbance levels (A = 0.252, $p < 0.001$) with significant pair-wise differences between. The plots in block

2 appear to differ in ordination space from other blocks both before and after treatments (Figure 2.6). No difference occurred between the ant community compositions in area of disturbance levels when block 2 was eliminated from analysis, suggesting that block 2 was largely influencing the results.

Based on the change in directional vectors by treatment from before to after harvest, the change in community composition was more variable in treatment plots than control plots. Differences in composition were also associated with the spatial distribution of the harvest disturbance. Treatments that had more spatially dispersed disturbance (for example, single tree selection had a more dispersed traffic of skid trails than group selection), varied more in ant composition following treatment than those with more concentrated disturbance areas (Figure 2.4). MRPP analysis of the NMS ordination scores indicated no significant difference between the relative positions in ordination space for pre-harvest and post-harvest data (Figure 2.6).

Seed Removal

In addition to fire ants, four species of ants were observed to remove or attempt collection of seed: *D. bureni*, *B. depilis*, *Pheidole* sp., and *P. dentata*. Regardless of year, fire ants were present at 65.52% of the seed removal trials and removed the majority of all seed, 80.93% (Before: df = 4, f = 43.94, $p < 0.0001$; After: df = 4, f = 24.82, $p < 0.0001$) (Figure 2.7). The next most frequent species in seed removal trials was *Pheidole* sp., occurring in only 10.34% of the first year trials and 15.52% of the second year trials (Figure 2.8). Seed removal rates did not differ among species of ants other than fire ants. There was no difference in seed removal due to treatments for native ants or fire ants (Native ant: df = 3, f = 0.47, $p > 0.05$; Fire ant: df = 3, f = 0.37, $p > 0.05$). Overall seed removal was greater the first year for only fire ants (df = 150, t =

0.1085, $p < 0.05$), but the greatest decrease in seed removal took place in the plot where no treatment was applied. Seed removal was not significantly different for the native ant species (df = 150, t = 0.7094, $p > 0.05$) between years. Between plots each year or between years, pitfall trap data did not reveal any differences in mean ant abundance or relative abundance for fire ants and native ants.

Past Disturbance

Native ants had a higher mean abundance in wiregrass-dominated plots relative to old field plots before the harvest (df = 46.044, t = -2.21, $p < 0.05$) and fire ant abundance did not differ. In comparing the relative abundances, the percentage of native ants was significantly higher in wiregrass plots and the percentage of fire ants was significantly higher in old field plots (Figure 2.9). However, this difference was a function of the low abundance of fire ants in block 2. Removal of block 2 from the analysis resulted in no difference in percentage of fire ants in wiregrass versus old field plots (Figure 2.10).

There was a significant difference before harvest in mean fire ant abundance between blocks (df = 5, f = 10.26, $p < 0.0001$). Block 4 and 5 (located in the same burn unit, but with different dominate ground cover) were different from each other. The less disturbed block 5 with a wiregrass-dominated understory had a significantly higher number of fire ants. For native ants there was also a significant block effect (df = 5, f = 2.41, $p < 0.05$) (Figure 2.11). A difference in total number of ants also occurred between blocks (df = 5, f = 4.54, $p < 0.05$).

The observed species richness was significantly higher in areas of wiregrass than in old field before the timber harvest (df = 1, $p < 0.05$). Block 2 had significantly higher species richness than all other plots before harvest, and this result was mirrored in all the estimates of species richness.

Ant composition differed among blocks prior to treatments (A = 0.367, $p < 0.001$ and this difference persisted after the treatment was applied (A = 0.386, $p < 0.001$). Table 2.4 shows the significant pair-wise differences in ordination distances. The ant community composition in block 2 was significantly different than all other blocks (Figure 2.6). Also, the pair-wise differences in ordination distances of block 1 (located in old field ground cover) were different from all wiregrass dominated blocks before harvest. Ant species composition was not significantly different between wiregrass and non-wiregrass plots before or after harvest.

Discussion

When fire ants invaded the area of the study, they became the most abundant ant in the ecosystem (Carroll and Hoffman 1997, Stuble et al. 2009). Although the reason fire ants were able to invade these forest interiors is unknown, it may have to do with previous undocumented soil disturbances or the continual disturbance of bi-annual burns (King and Tschinkel 2008). The results obtained in this study indicate that the differences in soil disturbance of the various timber harvest techniques had no effect on ant abundance and few effects on the community composition and species richness. This finding is consistent with another report that the ant community is resilient to some degree of soil disturbance (Andersen et al. 2009). In contrast, studies of intense disturbance by clear-cutting harvests performed in summer have shown to greatly reduce the species richness and increase the abundance of fire ants (Zettler et al. 2004). In our study, the lack of a significant ant community response was most likely due to a lesser degree of disturbance in the canopy and ground cover associated with a timber harvest of selected trees (Andersen et al. 2009). Soil disturbance in our study was not great enough to completely disrupt the rhizomes of the perennial vegetation, and in the spring many of the sites of exposed soil were quickly re-vegetated. Harvesting in the winter, when most ant species are not foraging or reproducing (Tschinkel 2006, Stuble et al. 2009), may have had a reduced impact on the ant community.

Natural fluctuations in weather and temperature complicate seeing a clear picture of the immediate ant community interactions with soil disturbance. The small initial changes in the estimated species richness and the potentially diverging variation in ant community composition may amplify in time. The altered soil and increased woody debris may shift ant species colonies in new locations and provide new habitat, changing the ant community composition. Also, in the group harvest treatment and group with retention treatment, the canopy cover was greatly decreased in concentrated areas. This open canopy may allow alates (winged reproductive ants) to enter in the canopy more easily. Based on the fact that fire ants have been observed to prefer open areas (Stiles and Jones 1998, King and Porter 2007) and quickly invade newly disturbed areas (Porter et al. 1988, Zettler et al. 2004), subsequent sampling will be important.

The finding of no difference in seed removal from treatment plots is consistent with the fact that the ant community showed little change in composition. Seed removal was performed by the same ant species both years fire ants remained the dominant ant to remove elaiosome-bearing seed regardless of treatment. The fact that fire ants removed fewer seeds post-harvest may be due simply to natural fluctuations in fire ant foraging (Tschinkel 2006). Although the frequency of fire ants visiting Petri dishes did not change between years, there was less fire ant recruitment of additional workers to remove seeds in the second year. Less recruitment reduced the total number of seeds fire ants removed. The removal rate by fire ants probably differs from that of *Solenopsis geminata,* the native fire ant largely displaced by the red imported fire ant that was a major seed remover and consumer (Hölldobler and Wilson 1990). An invasive fire ant colony does not consume as many seeds as part of their diet as that of a *S. geminata* colony (Tennant and Porter 1991), but invasive fire ants may fulfill a similar role as a seed remover. Although fire ants may be the main seed removers, native ants are still able to collect some seeds, perhaps maintaining some degree of historical dispersal of seeds.

Our findings suggest past disturbance is only one factor in determining the extent of fire ant invasion into the longleaf ecosystem and the assemblage of the ant community. For example, in two areas where the only difference was dominant ground cover, the wiregrass dominated area contained more fire ants in abundance than the other one dominated by old field vegetation. This is contrary to the hypothesis fire ants prefer more disturbed areas (King and Tschinkel 2008). Also, an area with old field vegetation and the lowest species richness actually contained some patches of wiregrass, indicating it was only partially historically disturbed. All the areas of this study varied in native and fire ant abundance independent of their dominate ground cover. Our findings clearly show fire ant abundance and native ant abundances are assembled independent of each other and independent of the ground cover in longleaf pine stands (Figure 2.11). Other factors, relating to the order of arrival, land management, and proximity to corridors of invasion (in the case of fire ants), may have a legacy in the ant community independent of wiregrass presence.

This study unexpectedly exposed an area of longleaf-wiregrass forest at Ichauway that had a distinctive ant community (Figure 2.6). The higher species richness in this area coupled with a low number of fire ants is perplexing given the similarity in soils, plant community, and the fact that plots were located around main transportation routes through the property.

Presumably, this proximity to roads would have facilitated the spread of fire ants (Stiles and Jones 1998). One possible explanation for the unique ant community in block 2 may involve a period of fire suppression (at least 8 years) that occurred in this block over 20 years ago. Whether or not the resulting encroachment of hardwoods that followed altered the ant community enough to have lasting effects is debatable, given that annual or bi-annual prescribed fires have been applied for the last 20 years (King 2004, Zettler et al. 2004). Even though many studies indicate fire ants do coexist with a diverse native ant community (Morrison 2002, Graham et al. 2004, King and Porter 2007, King and Tschinkel 2008, Stuble et al. 2009), this area with a small population of fire ants and an increased species-rich ant community suggests that competitive interactions may occur at some scales.

The most abundant native ant species in the study site do coexist with fire ants with the exception of *P. dentata*, which was more likely to be found in areas with a low density of fire ants. Similar to fire ants, this species is also omnivorous, similar in size, and invades areas of newly exposed land. Thus, these two species may be in more direct competition over food resources than other ant species (Zettler et al. 2004).

Our finding that the predatory thief ant, *S. carolinensis* (Hölldobler and Wilson 1990), was more likely to be found in areas with fire ants contrasts with that of Stuble et al. (2009) in which an inverse relationship between fire ants and *S. carolinensis* was reported. This discrepancy may simply be due to locational and seasonal fluctuations. Even so, it seems to provide additional support that native ant species colonization is independent of fire ant population densities.

The initial resilience of the native ant community contributes important information relative to understanding impacts of ecological forestry practices on ecosystem processes. Also, areas dominated by fire ants would not experience a sudden increase in fire ants for the low intensity harvest techniques used in this study. The possible long term effects on the ant community due to reduced canopy conditions and the altered landscape need to be pursued to determine lasting effects from soil and canopy disturbances. In addition, further examination of landuse history across the landscape at Ichauway relative to fire ant composition may reveal legacy effects that influence the susceptibility of a site to fire ant invasion and other native ant community differences.

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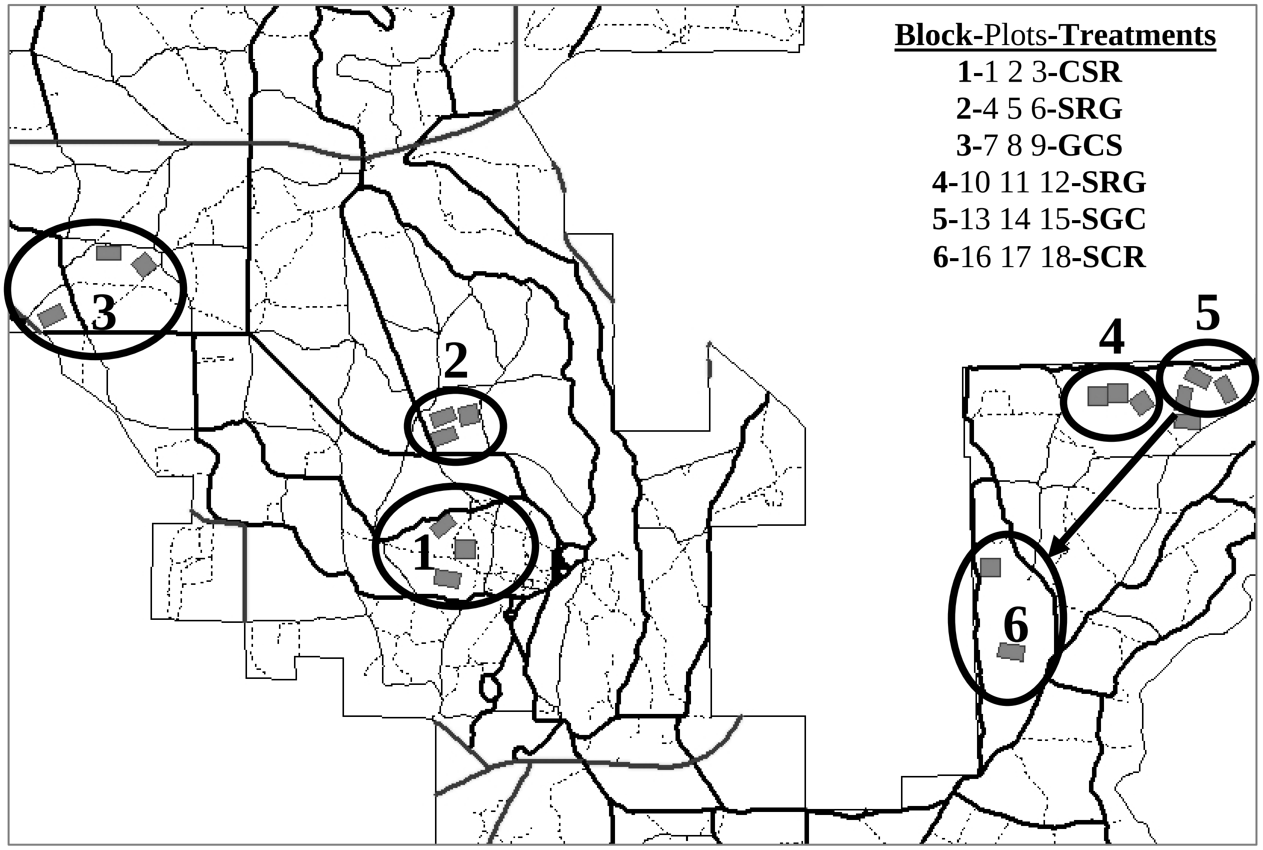

Figure 2.1 Each square is one plot and the circle around them with the number indicates the block. One plot close to block 5 is actually in block 6, indicated by the arrow. Plot numbers correspond to treatments within each block and are coded as S: single tree selection, C: un-harvested control, G: group selection, and R: group selection with retention.

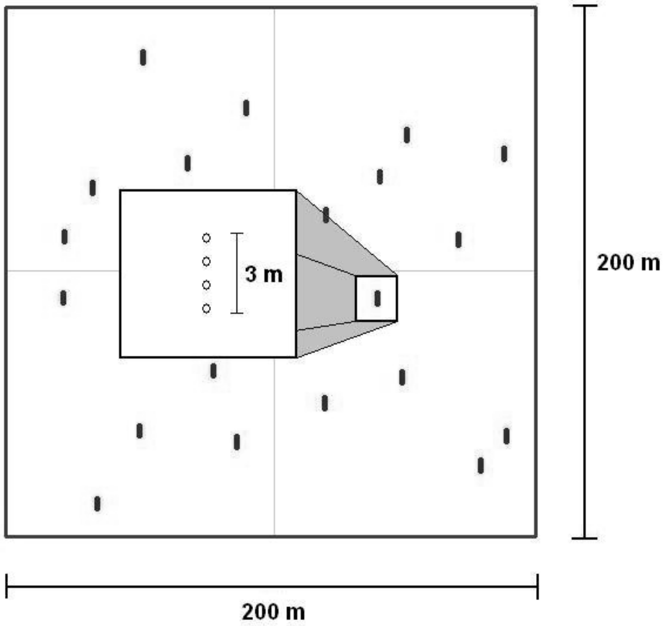

Figure 2.2 Example layout of pitfall traps within plots. Each circle in the zoom-in view is a single pitfall trap, and all four traps at each site are pooled into a single vial.

Table 2.1 The criteria for determining the soil disturbance classes.

	Area of Disturbance	Number of Skid Trails	Number of Traps in Disturbance
Low	3000-5000m^2	<100	6-8
Medium	5000-7000m^2	100-300	9-11
High	7000-9000m^2	>300	12-13

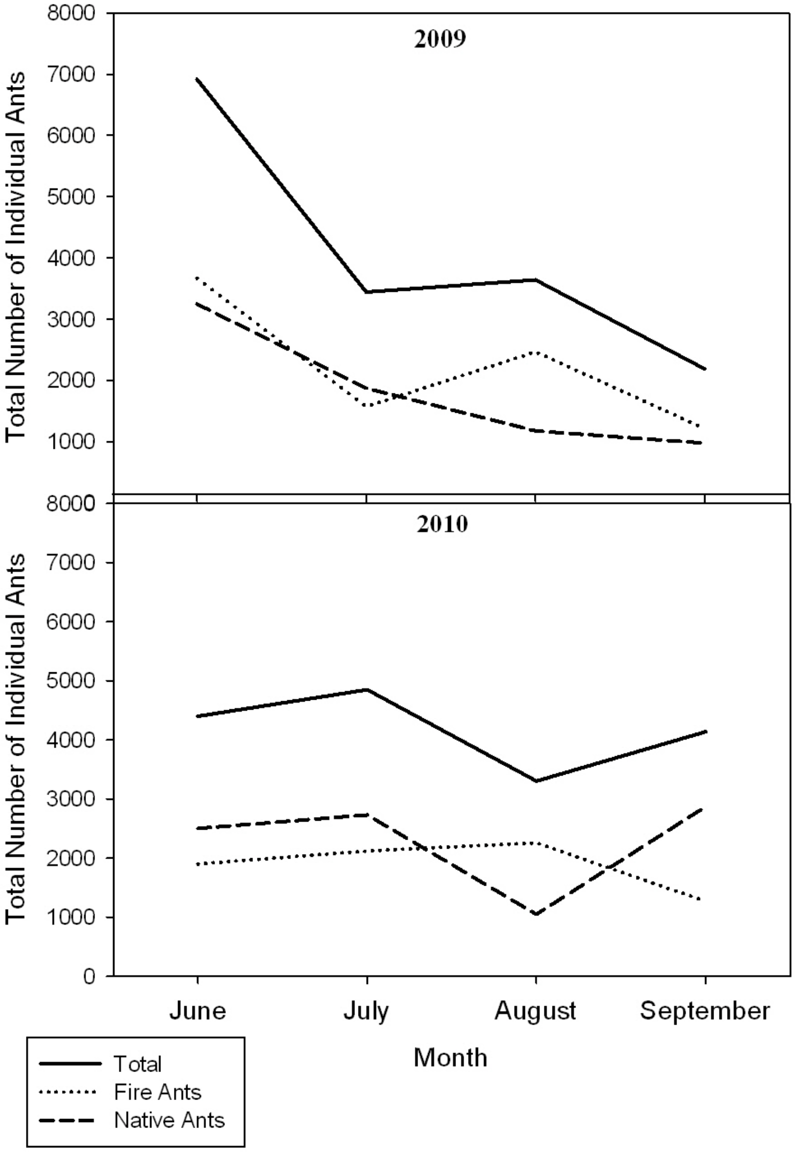

Figure 2.3 The seasonal variation in the number of individual ants captured before and after the timber harvest.

Table 2.2 The analysis of covariance involving all types of disturbance groupings. (degrees of freedom = 3, significance level = 0.05)

		f	**p**
Harvest Treatment	Fire Ants	0.54	0.6600
	Native Ants	1.41	0.2483
	Total Ants	1.25	0.2990
Area Disturbed	Fire Ants	0.88	0.4548
	Native Ants	0.63	0.6008
	Total Ants	0.67	0.5733
Number of Passes	Fire Ants	0.53	0.6664
	Native Ants	0.66	0.5805
	Total Ants	0.15	0.9315
Individual Traps	Fire Ants	0.15	0.9284
	Native Ants	1.10	0.3563
	Total Ants	0.66	0.5794

Table 2.3 Probability and odds ratio of individual ant species presence with fire ants based on logistic regression. Odds Ratio = ratio of the odds for each ant species to be found with fire ants to the odds without fire ants.

Ant Species	**Odds Ratio**	**Probability**
Brachymyrmex depilis	4.187	0.783
Dorymyrmex bureni	1.995	0.749
Pheidole dentata	0.436	0.444
***Pheidole* sp.**	0.606	0.538
Solenopsis carolinensis	0.540	0.516

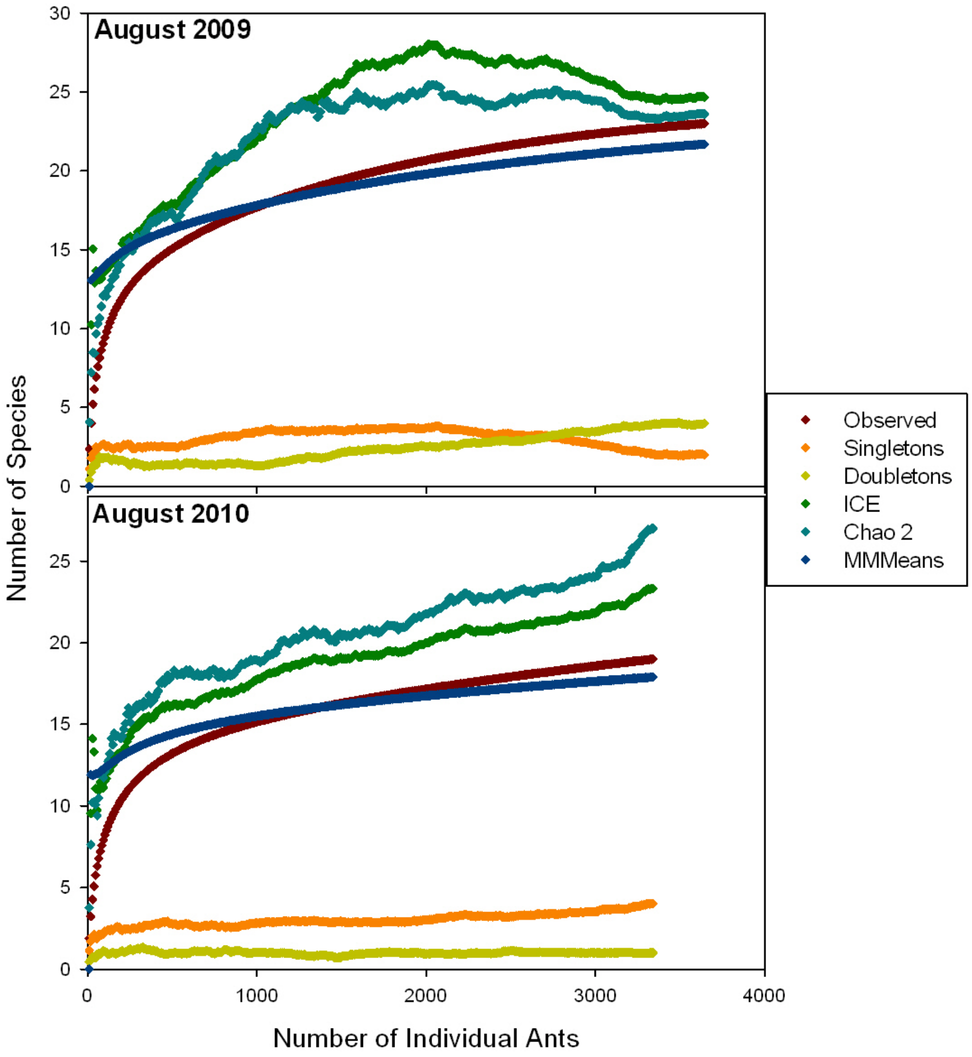

Figure 2.4 Species accumulation curves (Observed) with estimates of species richness (ICE, Chao 2, and M-M Means) from the under-sampled month (August 2010) and a representative month that was adequately sampled (August 2009). Singletons and Doubletons (the lower two lines) were also included because they are used to calculate the nonparametric estimators ICE and Chao 2. Note how estimates of species richness by ICE and Chao 2 begin leveling off in August 2009.

Table 2.4 Significant pair-wise differences between blocks based on ant species composition with inclusion of block 2. A is the chance-corrected within group agreement for which high values indicate less variation within groupings of plots (e.g., A = 1 occurs when all plots are identical within a block, and A = 0 when the variation within blocks is equal to that expected by chance).

Before Harvest

Block Comparison	**A**	**p**
1 vs. 2	0.429	0.022
1 vs. 4	0.413	0.022
1 vs. 6	0.302	0.026
2 vs. 3	0.349	0.025
2 vs. 4	0.429	0.022
2 vs. 5	0.429	0.023
2 vs. 6	0.413	0.023
4 vs. 6	0.302	0.024

After Harvest

Block Comparison	**A**	**p**
1 vs. 2	0.429	0.022
1 vs. 4	0.365	0.024
2 vs. 3	0.429	0.023
2 vs. 4	0.429	0.023
2 vs. 5	0.429	0.022
2 vs. 6	0.429	0.022
4 vs. 5	0.206	0.032

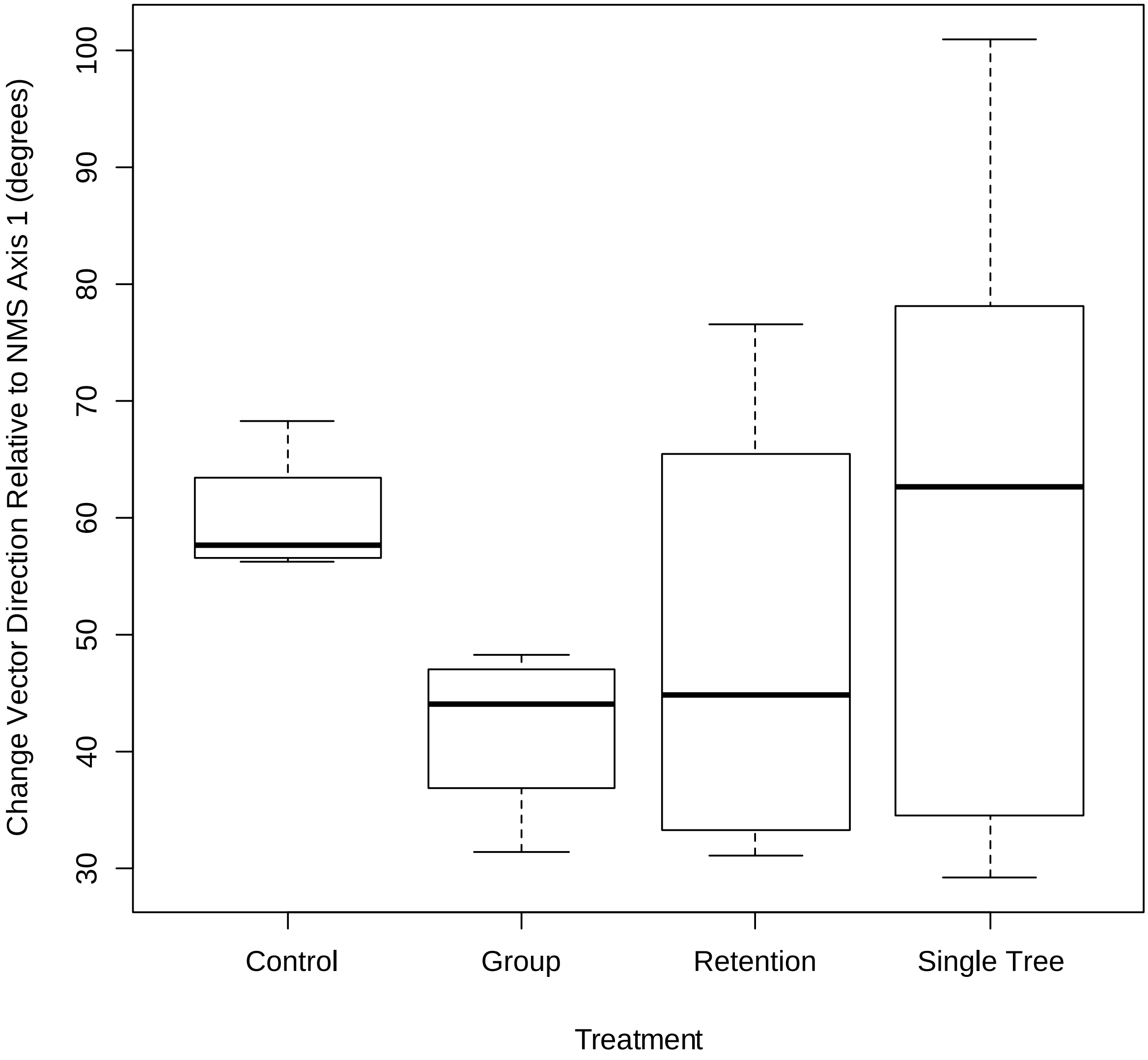

Figure 2.5 Change in vector angles (degrees) relative to NMS Axis 1 by treatment group. Note increased variation in treatments compared to the control, especially in group with retention and single tree selection treatments.

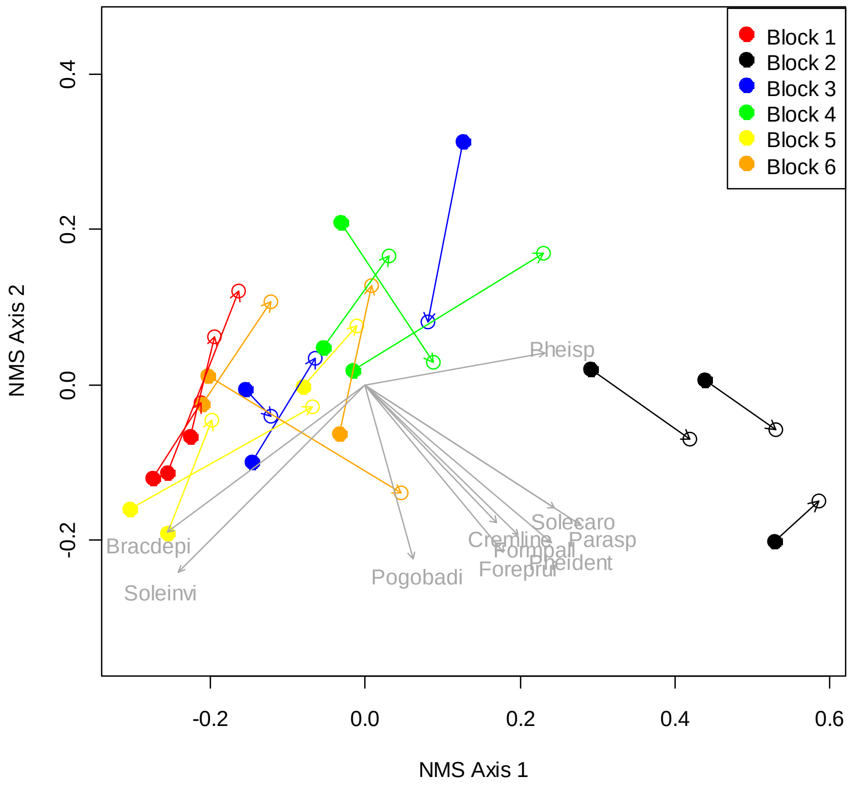

Figure 2.6 The location of plots in the 2-dimensional NMS ordination space by treatment group at pre- and post-harvest. Samples in close proximity have more species in common, while more distant samples have less species in common. The arrows go from before to after harvest for each individual plot. Note block 2 plots (black circles) are separate from all other plots.

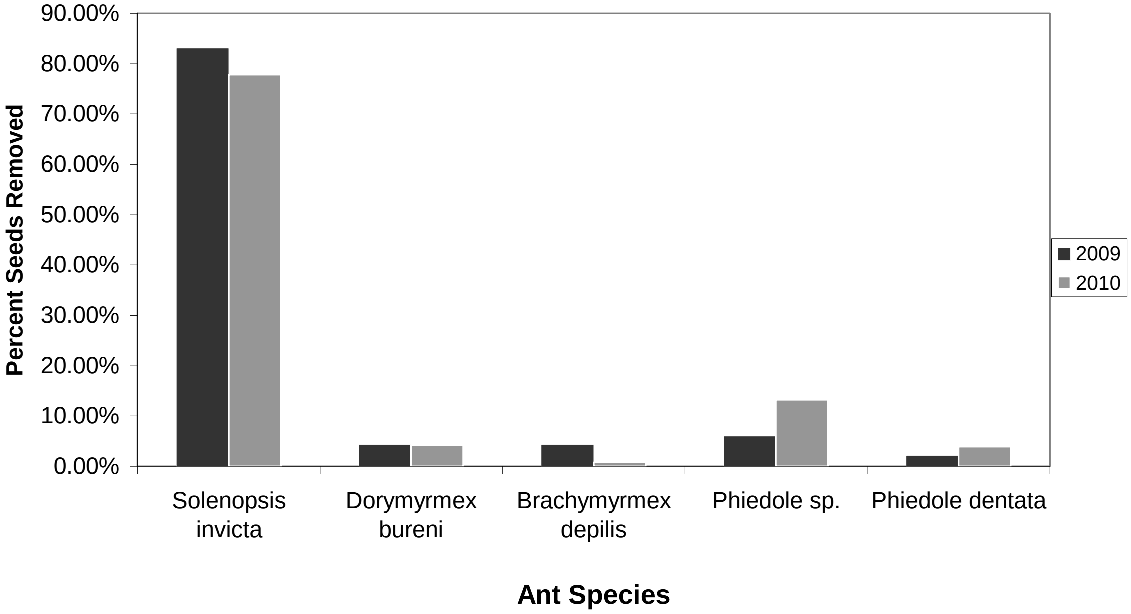

Figure 2.7 The percent of seeds removed by each ant species out of the total amount of seeds removed.

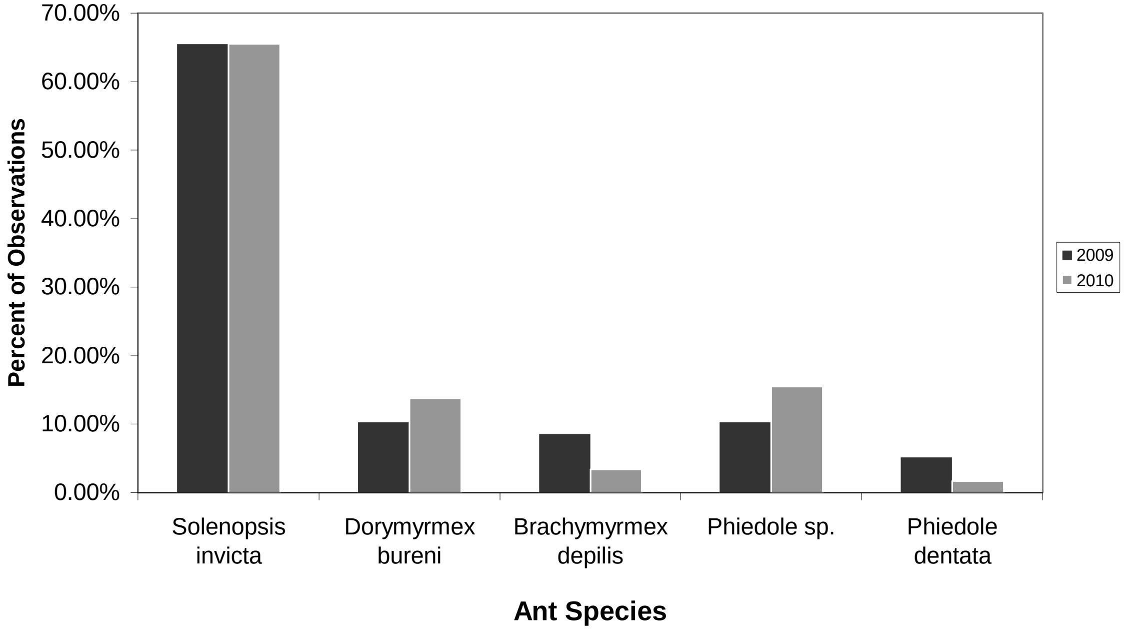

Figure 2.8 Percentage of observations in which an ant species removed seed.

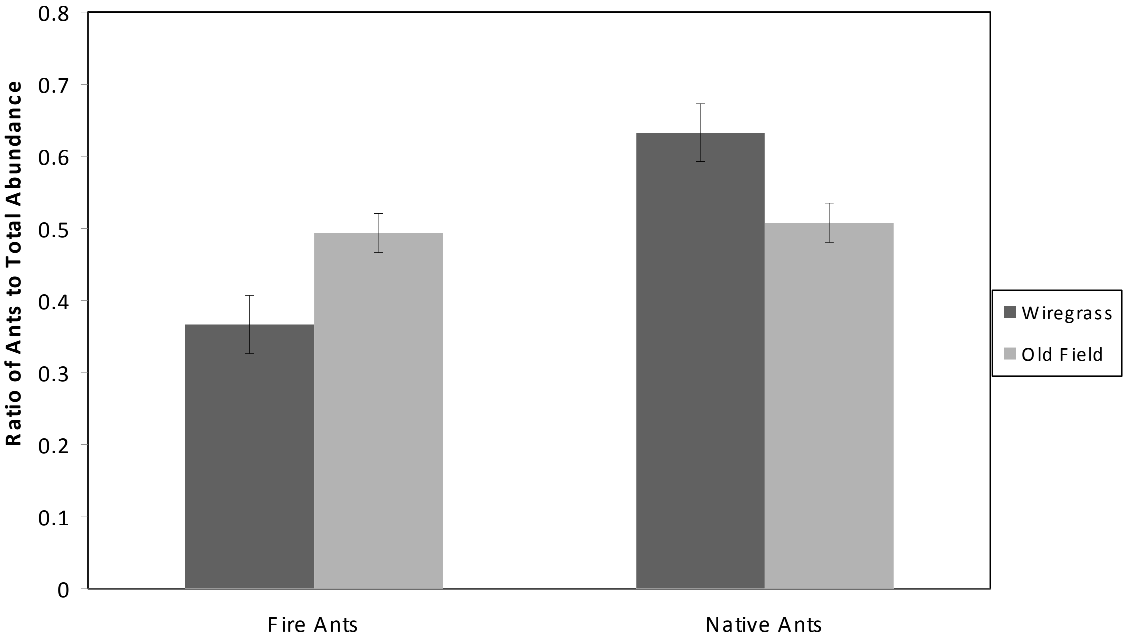

Figure 2.9 The relative abundances of fire ants or native ants in historically disturbed (Old Field) or undisturbed (Wiregrass) longleaf pine forest.

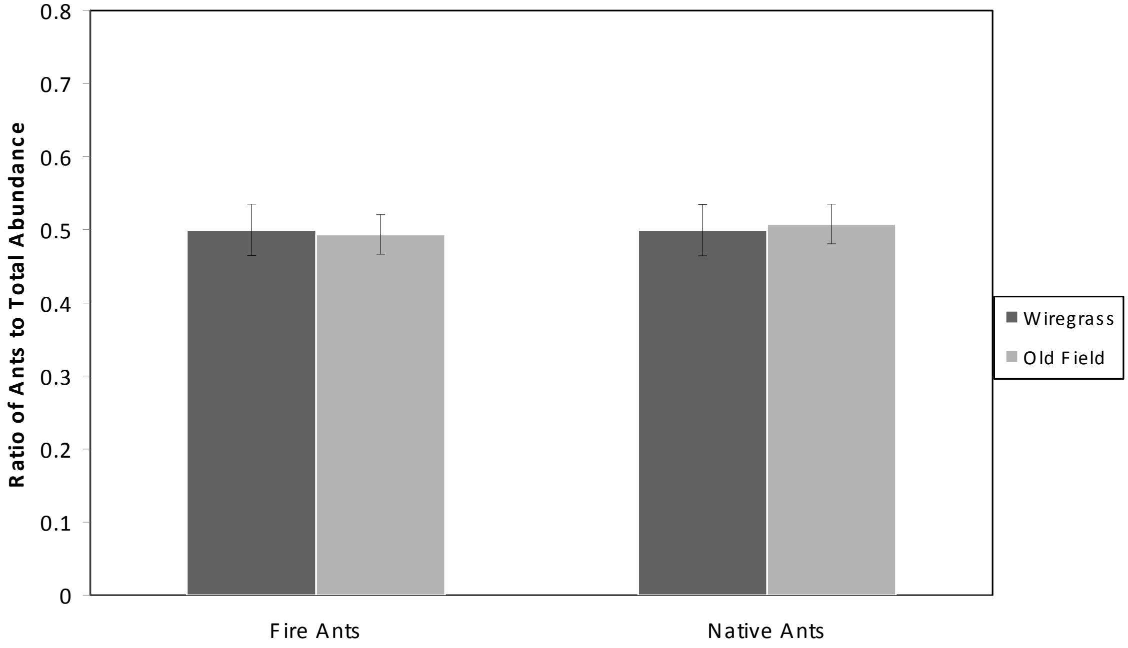

Figure 2.10 The relative abundances of fire ants and native ants in two different ground cover types that may reflect past disturbances without the outlier area, block 2.

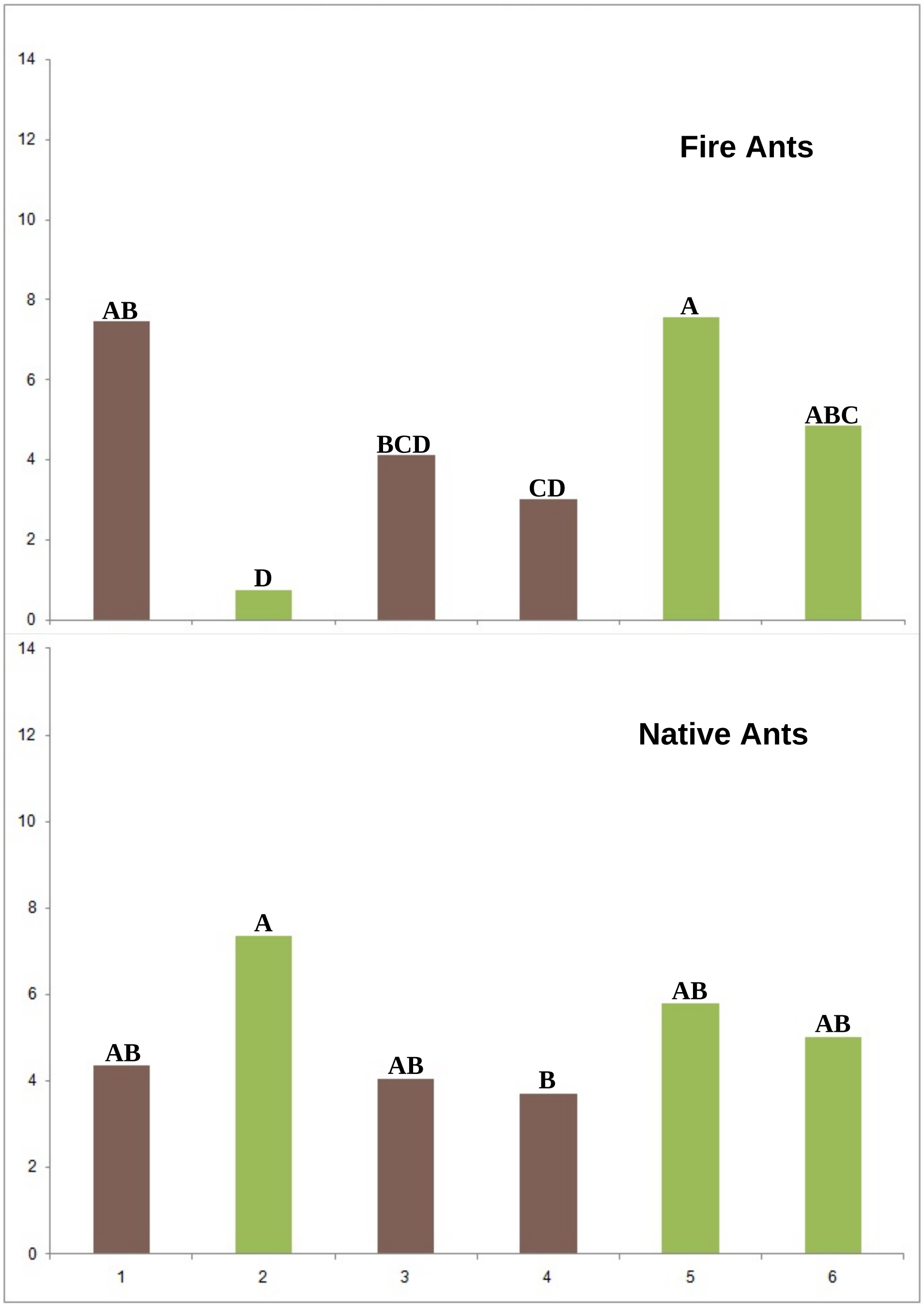

Figure 2.11 Comparison of mean ant abundance per vial by plot between blocks. Green (light) indicates wiregrass presence and brown (dark) indicates old field ground cover. Similar letters on bars indicate no significant difference (e.g. all bars with A are not significantly different, all bars with B… etc.). Significant differences were evaluated with a statistical block on the month the sample was collected which is not reflected in bar values.

CHAPTER 3

THE EFFECTS OF THE RED IMPORTED FIRE ANT ON SEED FATE IN THE LONGLEAF PINE ECOSYSTEM[2]

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[2] M.E. Shearin. To be submitted to *Plant Ecology*.

Abstract

The dominance of the red imported fire ant (*Solenopsis invicta*) in the southeastern United States has led to questions of its role in disrupting natural patterns of myrmecochory, or if it actually performs some degree of seed dispersal. We examined the seed fate of ant dispersed seed in the longleaf pine ecosystem. First, we determined to which seeds fire ants were most attracted by recording removal rates. Then we examined the final location of removed seeds by using a wax cast to examine the nest contents, and locating dyed seeds deposited in trash piles on the ground surface. Finally, we determined if the germination rate of seeds deposited by fire ants was affected.

Fire ants were most attracted to myrmecochorous seeds with elaiosomes. They collected small legume and grass seeds without elaiosomes at a much lower rate. The wax casting of the fire ant nest did not reveal any seeds stored in the chambers. Of the dyed seeds presented to the fire ants, we recollected 30 to 40% of them in trash piles. The undiscovered seeds were considered destroyed or buried in foraging tunnels. A small percentage of the deposited seeds were able to germinate, but there was no difference in percent germination from either species between seeds manipulated by fire ants and the control. This low germination was most likely due to using a high percentage of immature seeds used in the study. Our findings show that fire ants are facilitating movement of seeds in the longleaf pine ecosystem.

Introduction

There are many species of plants that depend on ants for seed dispersal (myrmecochory) (Sernander 1906, Lengyel et al. 2010). This mutualism is not restricted to a particular species of ant, but instead on the carnivorous and omnivorous ants in general (Hughes et al. 1994).

Typically, ant-dispersed seeds have an elaiosome, an appendage that contains fatty acids similar to insect hemolymph and are fed to ant larvae (Berg 1966, Beattie 1985). Once the elaiosome is removed, the ants are no longer interested in the seed and deposit it into nutrient-rich trash piles either above or below ground. Underground middens may protect the seed from fire or rodent predation (Berg 1975, Reichman 1979). In addition, the seeds are moved away from the mother plant, which may increase survival probability (Andersen 1988).

This mutualistic relationship exists in many habitats worldwide (Rico-Gray and Oliveira 2007). In areas of disturbance, myrmecochorous plants sometimes are slow to migrate back into restored habitats (Matlack 1994, Kirkman et al. 2004, Ness and Morin 2008), and the degree to which invasive ants may be affecting this mutualism by preventing the dispersal of seed has been questioned (Kirkman et al. 2004). A disruption of dispersal processes could occur in two ways: the invasive ant can disrupt the native ant community so that the seed dispersers are no longer in abundance to disperse as many seed (Porter and Savignano 1990, Christian 2001, Holway et al. 2002), or the invasive ant can move seeds directly and alter the fate of the seed.

The red imported fire ant (*Solenopsis invicta*) invaded the southeastern United States in the 1930s (Calcott and Collins 1996). This aggressive, omnivorous ant is very abundant in most of its invaded areas, but coexists with much of the native ant community (Stuble et al. 2009). Indirectly, fire ants have affected folivory by eliminating insect herbivores (Stiles and Jones 2001), but fire ants were also observed to affect elaiosome-bearing seeds directly by taking them into foraging tunnels (Carroll and Hoffman 1997, Ness 2004, Stuble et al. 2010). Once seeds are moved underground, the fate of the seed, including its ability to survive and germinate, is unknown. Fire ants may deposit seeds underground, place the seeds in surface middens, or simply destroy the seeds.

In this study we examine how fire ants are directly affecting seeds by observing the effects of fire ants on native seed removal. Specifically, we address the following questions: 1) Which plant species or types of seed are preferentially removed by fire ants? 2) Are seeds removed by fire ants deposited above ground (into trash piles) or below ground (into nests)? 3) Does ant manipulation alter germination success of elaiosome-bearing seed?

Materials and Methods

Study Site

This study was conducted at the Joseph W. Jones Ecological Research Center at Ichauway, located in southwestern Georgia (Baker County). Ichauway maintains approximately 12,000 hectares of land dominated by second-growth longleaf pine (*Pinus palustris*) forest (approximately 90 years old), scattered agricultural fields, isolated depressional wetlands, and hardwood riparian corridors. Within the longleaf pine stands, the presence of species-rich native ground cover and the dominance of wiregrass (*Aristida stricta*) are indicative of sites that have never been disturbed by cultivation (Myers 1990). The property is located on karst topography, and the sandy soils range from well to poorly drained. The average daily temperature is 11ºC during winter and 27ºC during summer with an average annual rainfall of 137 cm per year (Georgia Automated Environmental Monitoring Network, University of Georgia). This property was historically managed for northern bobwhite quail with frequent prescribed fire for many decades. Currently, prescribed burns are carried out at two-year return intervals. Over 30 native ant species have been documented in the longleaf pine forests at Ichauway (Carroll and Hoffman 1997, Stuble et al. 2009). Although the exact date of arrival of fire ants is not documented, they were present in the county by the mid-1960s (Callcott and Collins 1996). Thus far, only the monogyne fire ant colonies have been discovered within the boundaries of the research center property. Although a list of the native myrmecochorous species is not complete, several elaiosome-bearing plant species are common and occur abundantly.

Seed Selection

To determine which plant species are preferred by fire ants, we set up a cafeteria-style experiment in which we presented fire ants with seeds of 27 native species and recorded the rate of removal in eight separate trials. Seeds were chosen from four main categories (composites, grasses, legumes, and elaiosome-bearing). At least five species from each category were tested overall. For species in which ants consistently had no interest, we discontinued further trials. This permitted a greater number of species to be tested, especially as the seasonality of native seeds changed and more species of seed became available.

To ensure fire ants were the main collectors of seeds, seed of all species were presented within a single fire ant territory where the fire ants would dominate food sources. We delineated territories by testing for hostility between fire ant workers from neighboring monogyne colonies (Tschinkel et al. 1995, Tschinkel 2006). Test tubes containing a small amount of tuna fish were placed at one meter intervals along eight lines radiating out from the fire ant mound, with one test tube placed directly on the mound. Once fire ants recruited to the bait, we moved each test tube to the one containing ants on the central mound to determine if the worker ants engaged in fighting. The farthest points along the lines from the mound where worker hostility did not occur were considered the territory boundaries. We placed Petri dishes within at least one meter from the outer boundary of the fire ant territory and at least one meter from the fire ant mound itself (Markin et al. 1975). Each Petri dish was also placed at least one meter away from each other. Fire ant forager density is approximately uniform within a territory except for a decrease around the mound, so fire ants were equally likely to discover each seed species (W. Tschinkel *personal communication*).

Seed presentation consisted of placing 10 seeds of each species in a separate Petri dish. To allow ants access to the seeds, and to exclude rodents, we melted 4 small holes into the dish rims and placed a lid on each dish. So that dishes containing seed species from the same category were not clustered around the fire ant mound, we interspersed them within the territory. Seeds were placed in dishes in the early morning or late afternoon when the ants were most active. We recorded the number of seeds remaining in the dish at half hour intervals for five hours.

Seed Fate

Although fire ants have been observed collecting seeds and taking them into foraging tunnels, the ultimate destination for these seeds is not known. Four possibilities exist: nest, surface, foraging tunnel, and/or destroyed. We first determined how many seeds were taken underground and, subsequently, how many seeds were found in midden piles on the soil surface. For seeds on the ground surfaces, we located a fire ant mound on level ground in a recently burned or low-vegetation area to maximize the ability to recover seeds following removal. To contain fire ants within the study area, and prevent other ant species from taking seeds outside the area, we constructed a 13.7 square meter enclosure around the ant mound by inserting 50.8 cm wide aluminum flashing into the soil. The flashing was inserted at a depth of 10 cm to cut through any ant foraging tunnels radiating out from the mound (Markin et al. 1975). We coated the top 3 cm edge of the aluminum flashing with Tanglefoot® (an insect trapping adhesive).

Within the enclosure, we placed two Petri dishes on opposite sides of the mound. One dish contained 100 *Piriqueta cistoides* (Turneraceae) seeds and the other contained 100 *Polygala grandiflora* (Euphorbiaceae) seeds, both are common elaiosome-bearing seed readily collected by ants. All seeds were coated in Rhodamide B dye which fluoresces in ultraviolet light. The dye does not affect fire ant collection of seeds, but the dyeing process enhances seed germination percentage and rate (the number of days required for germination) (*preliminary experiments*). We presented seeds to ants in the afternoon of the same day we constructed the enclosure to reduce the possibility that fire ants would dig underneath the flashing (W. Tschinkel *personal communication*). Five hours after presentation of seed, and for three to seven days after, we examined the enclosure area using an ultraviolet flashlight to locate ant trash piles containing deposited seed. If a midden was discovered, we collected the seeds. We microscopically examined each seed to determine if it was physically damaged and if the elaiosome was removed.

Next, we examined the germination rates of retrieved seeds with that of dyed seeds that had not been presented to ants. The un-presented seed was subjected to three treatments: elaiosomes removed, scarification, or un-manipulated control. For the scarification treatment we used a seed scarifier with 150A grit paper for three seconds. Seeds were planted in trays of potting soil and placed in a greenhouse, watered regularly with RO water. The number of germinants was recorded weekly for 10 weeks.

In addition to looking at seeds deposited on the surface, we examined the contents of the nest to determine if seeds were deposited in chambers. This was done by constructing a wax cast of the nest and excavating it using methods described by Tschinkel (2009). To construct the cast, we first removed the mound and vacuumed up dirt to reveal the major tunnels into the nest.

Then we poured melted paraffin wax until the nest was full (about 2 kg). We used three different colors of wax to organize extracted pieces by depth. After the wax solidified, we excavated the wax cast down to 110 cm and removed it from the ground. Each 10 cm section of the cast was melted, filtered, and examined for seeds.

Data Analysis

For the species removed, the rate of removal (calculated as the slope of the linear regression of seeds removed per half hour) was compared between species (Hughes and Westoby 1992). We also compared percentage of seeds removed per five hour trials by species. For both comparisons we used an analysis of variance (ANOVA), blocking by trial (PROC GLM, SAS version 9.2). To test for differences in seed removal between all species with and without elaiosomes, we used a t-test (PROC TTEST, SAS version 9.2).

The percentage of presented seed taken underground, and then cast into midden piles was compared over time to determine if there was a seasonal effect on seed deposition. Percentages of elaiosome removal and retention of elaiosomes were determined as well. Percent seed germination was compared between seed treatments using an ANOVA (PROC GLM, SAS version 9).

Results

Seed Selection

For all species tested, seeds with elaiosomes were removed at a much higher rate (0.1879 seeds removed/half hour) and a greater percentage (58.9%) than seeds without elaiosomes (0.002 and 6.3%) (Table 3.2). Although a subset of grass and legume species were also removed, this occurred less frequently than removal of elaiosome-bearing species (Table 3.1). All of the composite species tested were completely ignored by the foraging fire ants.

Of the elaiosome-bearing species, *P. cistoides* had the highest rate of collection (0.7062) followed by *P. grandiflora* (0.5631) and *Viola sororia* (0.1787) (Figure 3.1). *Cnidoscolus stimulosus* had an intermediate removal rate and percent of seeds removed (Figure 3.2). Removal rates of *Croton argyranthemus* (0.0162) and *Stillingia sylvatica* (0.001) were similar to that of non-elaiosome-bearing seeds.

Seed Fate

We recovered an average of 36% of seeds presented. The mean number of seeds deposited by fire ants did not vary seasonally; however, the rate of removal and deposition decreased as the temperature dropped towards the end of the growing season. Of the seed removed and taken underground, approximately 41% of *P. cistoides* seed and 31% of *P. grandiflora* seed were redeposited on the soil surface. We assume seeds not found on the surface were either destroyed or deposited within the nest or foraging tunnels at a depth too deep to germinate. However, no seeds were found within any layers of wax extracted from nest chambers.

The majority of *P. grandiflora* seeds retrieved from the midden piles still had elaiosome attached and had evidence of slight markings by ant mandibles. In contrast, retrieved *P. cistoides* seeds usually had no attached elaiosome (Figure 3.3). We occasionally found detached elaiosomes of both species deposited in midden piles. Germination of ant-deposited seeds retrieved from middens did not differ from the control seeds, scarified seeds, or seeds with elaiosomes removed (*P. cistoides*: df = 3, f = 2.52, $p > 0.05$; *P. grandiflora*: df = 3, f = 0.45, $p > 0.05$) although overall germination was low.

Discussion

Seed dispersal by fire ants is biased toward their preference for seeds with elaiosomes (Ready and Vinson 1995, Zettler et al. 2001, Garrido et al. 2002). The amount of seeds they collect are based more on their immediate dietary needs than any granivorous tendency (Cassill and Tschinkel 1999). The removal and dispersal of elaiosome-bearing seed by the invasive fire ant likely differs from that of the native fire ant (*Solenopsis geminata*), which the red imported fire ant displaced (Wilson 1951). Typically, *S. geminata* is considered a seed predator storing seeds in chambers and then consuming the endosperm, whereas the invasive fire ant is not considered to be a seed predator (Porter et al. 1988, Tennant and Porter 1991). Even though *S. geminata* occurred in much fewer numbers than the invasive fire ant in the southeastern United States, the amount of seed collected by *S. geminata* far exceeded that of fire ants on a per colony basis (Risch and Carroll 1986, Hölldobler and Wilson 1990). In a study in Mexico (Risch and Carroll 1986), seed predation by *S. geminata* was found to be a significant factor influencing plant community composition.

Fire ants tend to collect smaller elaiosome-bearing seeds more readily than large ones, presumably because they are able to lift them. This would explain the relatively quick removal of the smaller seeds *P. cistoides*, *P. grandiflora*, and *V. sororia*. Although fire ants were initially attracted to the large-seeds of *C. argyranthemus*, after a few weeks the elaiosomes turned black and fire ants were no longer interested in them suggesting the elaiosome may become less attractive as the seed ages (Kjellsson 1985). Fire ants were occasionally attracted to *S. sylvatica*, the largest seed tested, but rarely attempted to move it. In contrast, fire ants recruited in large numbers to move *C. stimulosus*, which was similar in size to *S. sylvatica*. Interestingly, both of these elaiosome-bearing seeds are from the same plant family (Euphorbiaceae). Possibly, the preference for *C. stimulosus* is related to the chemical composition of the elaiosomes.

Although we found no evidence of granivory storage chambers within the nest, it is highly likely that seeds are left scattered within the foraging tunnels. Seasonal differences may be a factor in seed deposition in nests, and we did not examine temporal differences in nest content throughout the year. Even if seeds remain underground in nests or most foraging tunnels, they are likely too deep to germinate unless exposed by soil disturbance.

We found fire ants were the main seed removers in this ecosystem (Chapter 2), and a portion of seed is placed into midden piles on the soil surface (Figure 3.4). In previous studies, fire ants were classified as poor dispersers and frequent destroyers of seed (Ready and Vinson 1995, Zettler et al. 2001, Ness 2004). We occasionally found seed fragments in midden piles, but the majority of the dispersed seed in this study appeared undamaged. Since the amount of seeds damaged by a colony of fire ants is less than the amount damaged by a colony of the native fire ants (Tennant and Porter 1991), the increased density and number of the invasive fire ant colonies would be the determining factor for the effect on seed fate.

We found both seed species in the same middens although numerous potential middens were present. This indicates that a central location where the majority of seeds are processed is likely. There was no difference in the time each species was underground before deposition in surface trash piles. Also, the difference in the presence or absence of elaiosomes post-deposition may offer some insight into how seeds are processed. Elaiosomes on *P. cistoides* are more fragile than the firmly attached elaiosomes of *P. grandiflora* (Berg 1975). Because of this difference, the time it takes to remove a *P. cistoides* elaiosome may be less than the time it takes to remove a *P. grandiflora* elaiosome. Since both seed species and detached elaiosomes were found after the same time period, fire ants may process seeds with elaiosomes with the same degree of manipulation. Elaiosome presence, type, and seed size, may be predictors of seed dispersal of a species by fire ants.

Fire ants are the dominant removers of elaiosome-bearing seed and are successfully dispersing seeds in the longleaf pine ecosystem. While we have demonstrated that a considerable proportion of the seed is deposited on the surface of the soil, the fate of non-dispersed seeds relative to seedling recruitment still needs to be determined.

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Table 3.1 All species tested for seed removal and the resulting rates and percent of seeds removed. Rate of removal was calculated as the slope of the linear regression of seeds removed per half hour. “Elaiosome” refers to elaiosomes-bearing seed.

Seed Species	Category	Number of Seeds Presented	Number of Seeds Removed	Percent of Seeds Removed	Rate of Removal
Piriqueta cistoides	Elaiosome	80	80	100.0%	0.4623
Viola sororia	Elaiosome	50	46	92.0%	0.1117
Polygala grandiflora	Elaiosome	80	67	83.8%	0.4341
Cnidoscolus stimulosus	Elaiosome	80	40	50.0%	0.1019
Croton argyranthemus	Elaiosome	80	16	20.0%	0.0162
Euphorbia pubentissima	Spurge	80	16	20.0%	0.0201
Paspalum notatum	Grass	70	14	20.0%	0.0202
Zornia bracteata	Legume	80	10	12.5%	0.0160
Crotalaria rotundifolia	Legume	80	5	6.3%	0.0066
Aristida stricta	Grass	80	4	5.0%	0.0043
Stillingia sylvatica	Elaiosome	50	1	2.0%	0.0010
Andropogon virginicus	Grass	10	0	0.0%	0
Coreopsis lanceolata	Composite	10	0	0.0%	0
Desmodium floridana	Legume	10	0	0.0%	0
Dichanthelium acuminatum	Grass	20	0	0.0%	0
Digitoria sp.	Grass	20	0	0.0%	0
Helianthus angustifolius	Composite	20	0	0.0%	0
Lespedeza angustifolia	Legume	20	0	0.0%	0
Liatris tenuifolia	Composite	10	0	0.0%	0
Lygodesmia aphylla	Composite	10	0	0.0%	0
Pediomelum canescens	Legume	10	0	0.0%	0
Pinus palustris	Pine	10	0	0.0%	0
Rudbeckia hirta	Composite	10	0	0.0%	0
Saccharum alopecuroides	Grass	10	0	0.0%	0
Sorghastrum secundum	Grass	10	0	0.0%	0
Sporobolus junceus	Grass	10	0	0.0%	0

Table 3.2 Seed groups presented to ants including the percent of removal.

	Seeds Presented	Seeds Removed	%
# Elaiosome-bearing	450	265	58.9%
# Grass	230	18	7.8%
# Legume	200	15	7.5%
# Composite	60	0	0.0%
# Non Elaiosome-bearing	540	34	6.3%
# Total	990	299	30.2%

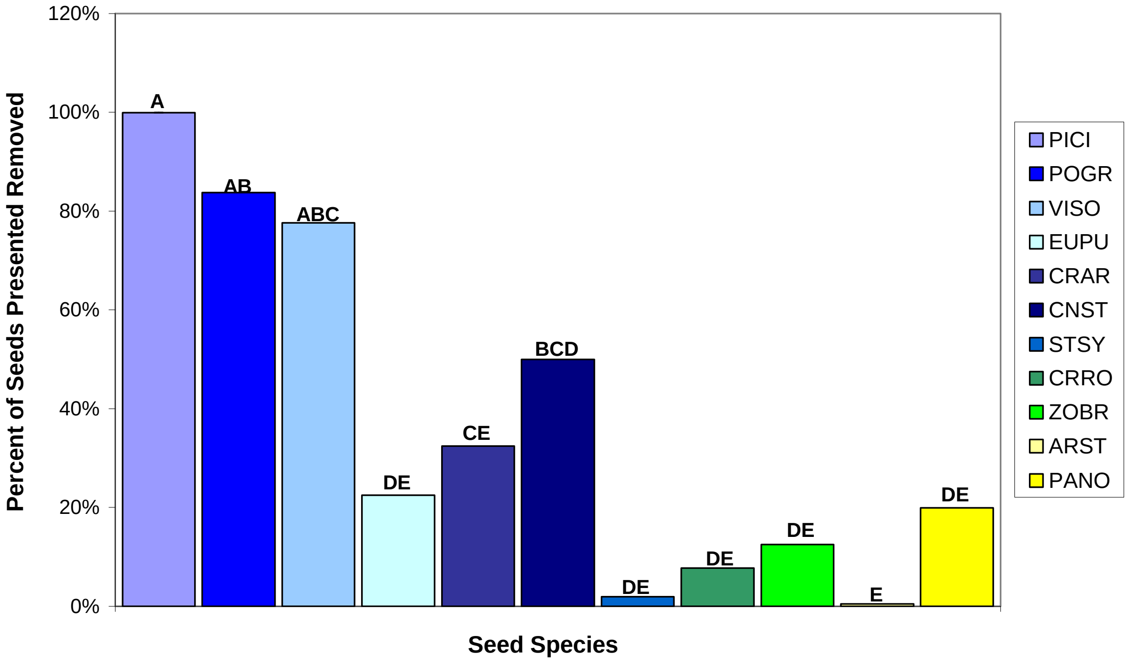

Figure 3.1 The percent of seeds removed for species that had seeds removed. Bars with the same letter are not significantly different. In the legend, species names are represented by the first two letters of the genus and species name (e.g. *Piriqueta cistoides* is PICI).

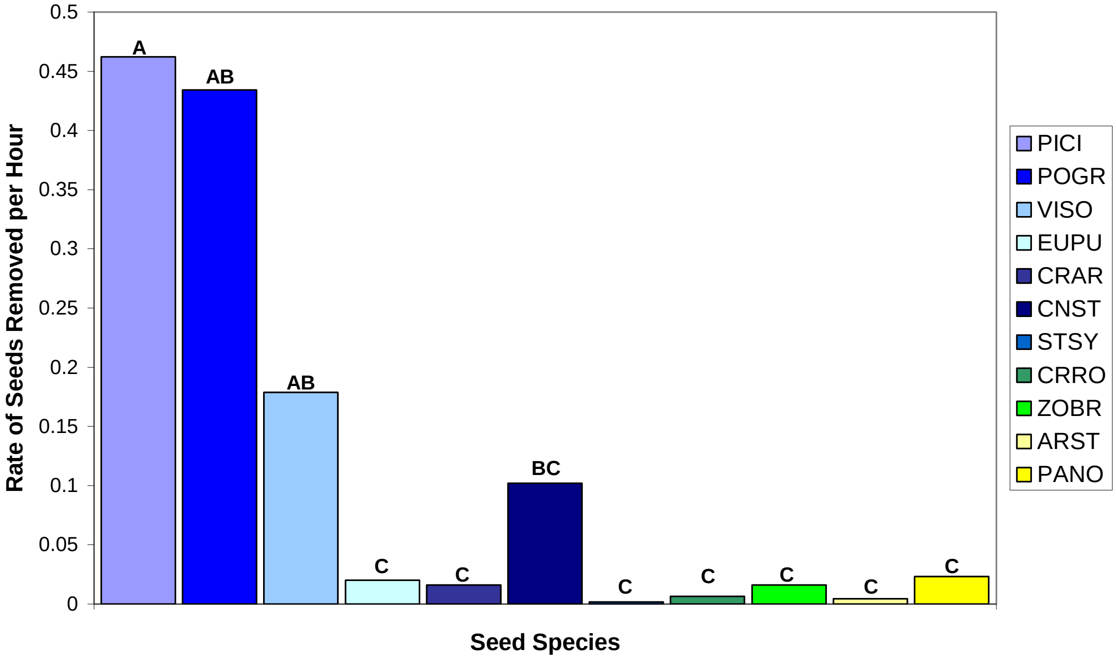

Figure 3.2 The rates of seed removal for species that had seeds removed. Bars with the same letter are not significantly different. Rate of removal was calculated as the slope of the linear regression of seeds removed per half hour. In the legend, species names are represented by the first two letters of the genus and species name (e.g. *Piriqueta cistoides* is PICI).

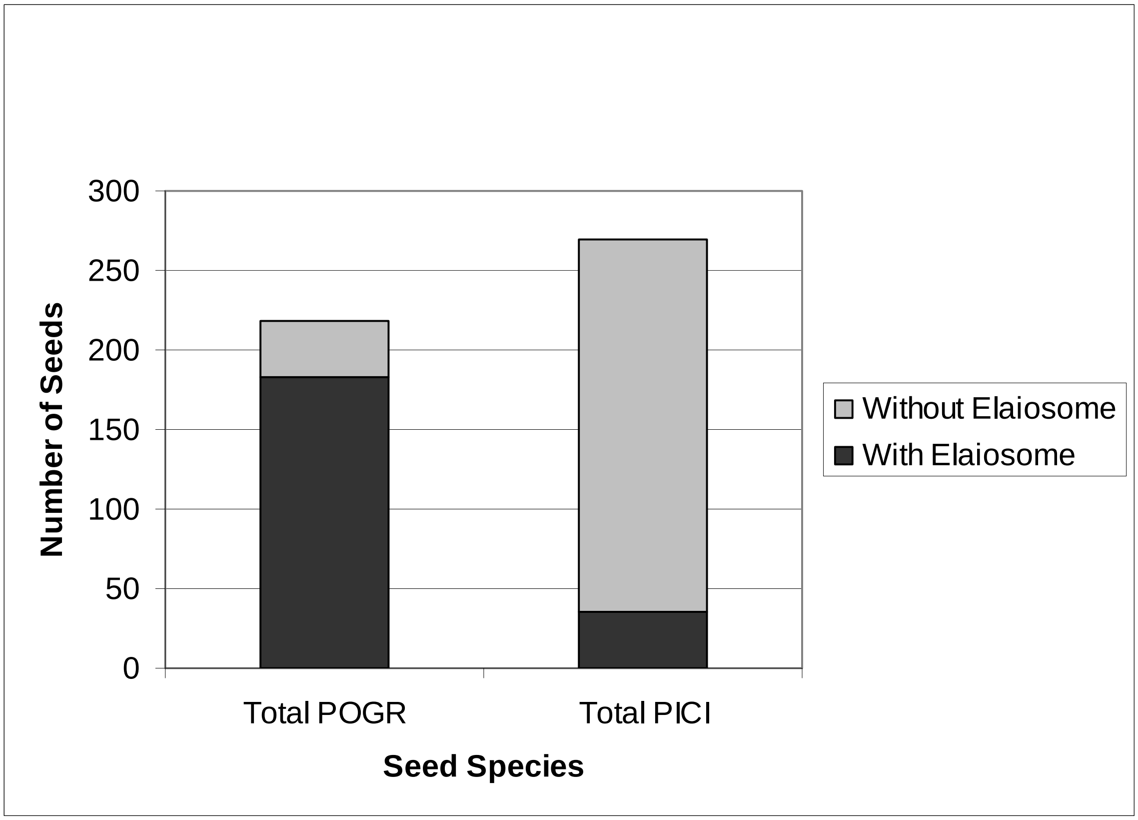

Figure 3.3 How many seeds of each species were recovered. The sections on the bars show how many of each seed had an elaiosome attached (dark) or removed (light).

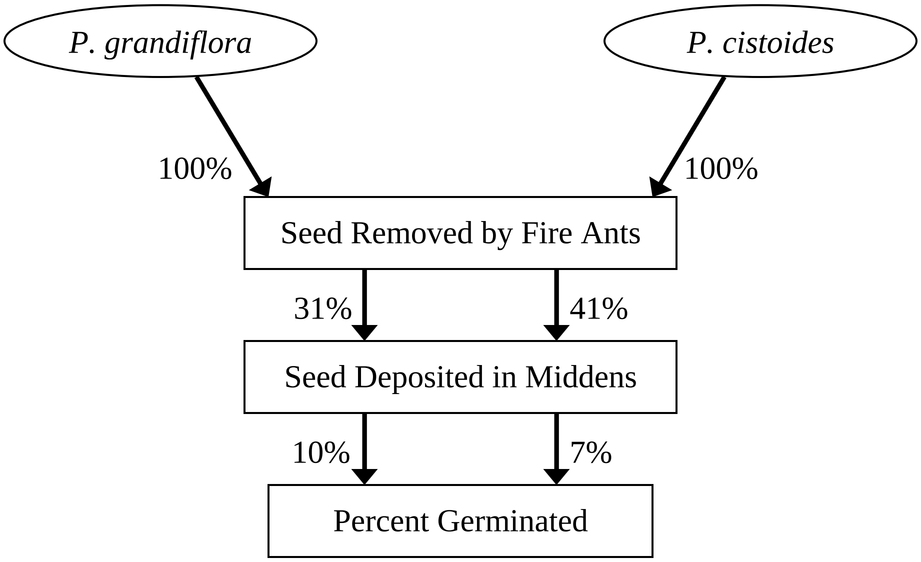

Figure 3.4 The pathway from seeds picked up by fire ants to percent germinated. Percentages for the box "Percent Germinated" are of the number of seeds deposited in middens instead of the original number of seeds removed.

CHAPTER 4

CONCLUSIONS

Chapter 2 Overview

The invasion of fire ants has had large economic and ecological impacts in the southeastern United States. Fire ants primarily invade highly modified environments, and thus impacts of fire ants are usually examined in the context of disturbed habitats. The invasion of fire ants into the longleaf pine ecosystem provided the opportunity to study the response of the native ant and native plant communities to fire ants; especially the effects that may be masked by disturbance in other areas. This study addressed the initial response of the ant community to a gradient of canopy and soil disturbances associated with timber harvest in a fire-maintained natural stand of longleaf pine. In Figure 4.1, we outline potential response of native and fire ants following disturbance that would address hypotheses regarding competitive interactions. Prior to treatment, the fire ants and native ant species maintain a population balance independent of each other. If a disturbance is intense or frequent enough to eliminate less tolerant species, this balance would shift. If the fire ant population increases, they may out compete native ants for food resources and prevent recolonization by native ants in disturbed areas. We also examined if a past disturbance, indicated by wiregrass presence, would have a lasting effect on the ant community.

Chapter 2 Results

Our findings demonstrated that in the areas already invaded by fire ants, further disturbance, such as timber harvest, did not increase fire ant populations or decimate native ant populations. In the conceptual model (Figure 4.1) the timber harvest disturbance would not be great enough to alter the balance between fire ant and native ant populations. This “no effect” may be due to the nature of the disturbance used in this study. The harvest techniques we used are not as devastating as some other types of timber harvest disturbances, such as clear cutting. None of the treatments damaged the soil or the plant community deeply enough to damage structure or root systems.

We also determined that the distribution of ants may be related to prior landuse, but we found no evidence indicating a direct relationship to soil disturbance that resulted in the removal of wiregrass. Although speculative, we suggest that other factors, perhaps relating to the order of arrival, land management, and proximity to corridors of invasion (in the case of fire ants), may have a legacy in the ant community independent of wiregrass presence.

Chapter 3 Overview

Fire ants are the main seed removers within this longleaf pine ecosystem. Potential outcomes of seed removal by fire ants include: 1) damage such that the seed can no longer germinate, or 2) deposition in a favorable or unfavorable location for germination (Figure 4.2). Deposition in unfavorable locations may eventually end in germination if the seeds are undamaged, and the soil is disturbed enough to bring seeds to the soil surface. Possible seed fates are laid out in the conceptual model (Figure 4.2). Specifically, this research addressed which seeds fire ants preferentially removed, if seeds were deposited in nest chambers or trash piles, and whether ant-deposited seeds can germinate.

Chapter 3 Results

We determined that fire ants were most attracted to seeds with elaiosomes and that these seeds were carried underground. While fire ants occasionally remove small grass and legume seeds, the fate of these seeds is unclear and the seeds may be consumed. Our investigations of seed fate of elaiosome-bearing seed provide no evidence that seeds are deposited in underground storage chambers or nests. Instead, we found that around a third of the seeds removed were deposited back onto the surface in midden piles surrounding foraging tunnel openings, but not necessarily adjacent to the same tunnels in which they were initially moved. Once seeds are deposited on the surface, a rain shower or further deposition by fire ants can rapidly bury them. Seeds not found in the nest or on the surface may be destroyed or incorporated into the tunnel structure. If not destroyed, these seeds would be too deep to germinate unless exposed by soil disturbance.

Of the seeds deposited on the surface, about 7 to 10 percent germinated when tested in greenhouse conditions. Though this germination rate is low, the percentage is not significantly different from any of the other treatments applied to the seeds, including the control. Low germination is most likely because of the immaturity of the majority of the seeds presented to ants. Therefore, fire ants do facilitate movement of seeds in the longleaf pine ecosystem to some degree due to the fact they do not destroy all seeds they collect.

Future Research

The timber harvest disturbances in this study opened up the tree canopy, especially in the treatments that created gaps (group and group with retention), which may facilitate ant migration into the area over time. Continued observations of the ant community would reveal if fire ants, which preferentially invade open areas, increase in relation to canopy gaps in the future. Also, the increased woody debris from branches left after harvest may increase habitat diversity, allowing habitat for additional species of native ants to become established.

Fire ants may not destroy all seeds they remove, but further research on seed fate would help clarify to what extent fire ants are assisting in seed deposition. Additional excavations of the fire ants nests and examinations of midden contents in all seasons would determine temporal differences in behavior of fire ants and whether deposition location changes. A seasonal examination of seed location after removal may determine the overall effect fire ants have on the seed bank. In addition to seed location, seed viability tests (Tetrazolium) of seeds at different stages of development, as well as seeds deposited by fire ants, would reveal a more accurate picture of germination by elaiosome-bearing seed before and after ant manipulation. Seed viability may also be a factor in the overall importance of ant dispersal for a given species. If seed viability is low, then fire ants collect mostly non-viable seed with little impact on overall seedling recruitment.

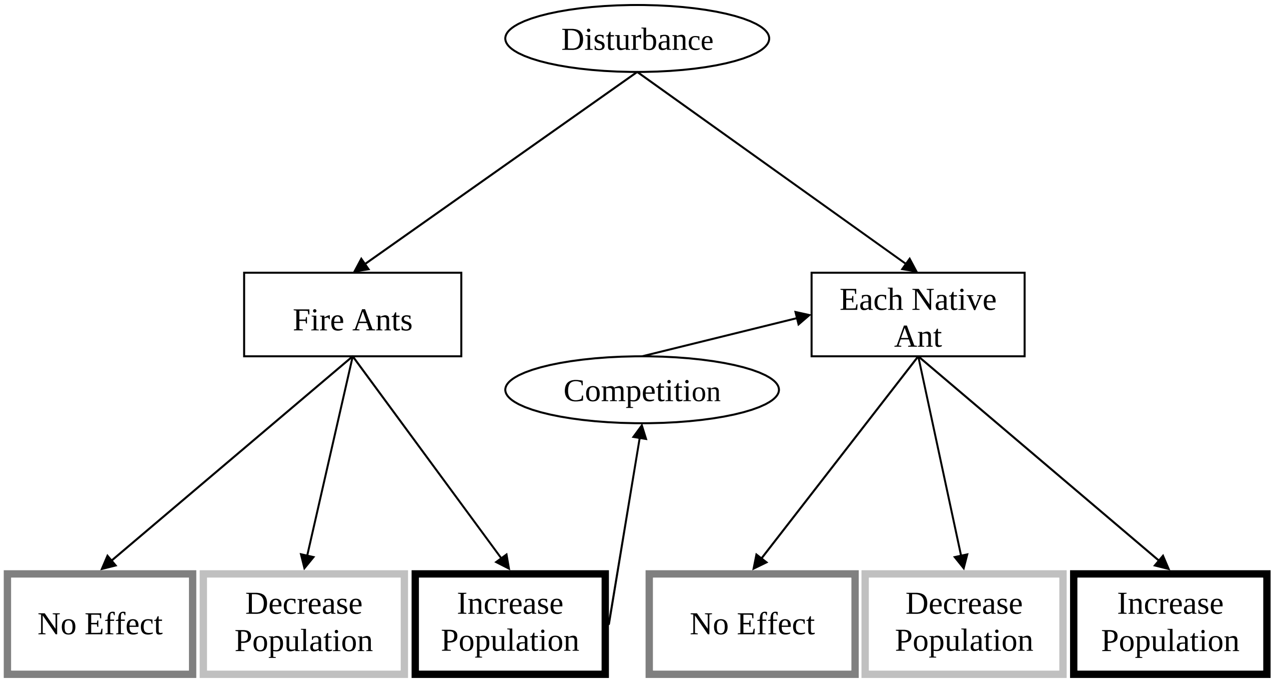

Figure 4.1 Disturbance potential outcomes when applied to areas with fire ants and native ants. Circles indicate actions performed, the boxes indicate the subjects of the actions, and shaded boxes indicate outcomes. The darker shade indicates increasing, lighter as decreasing, and the shading between darker and lighter as having no change.

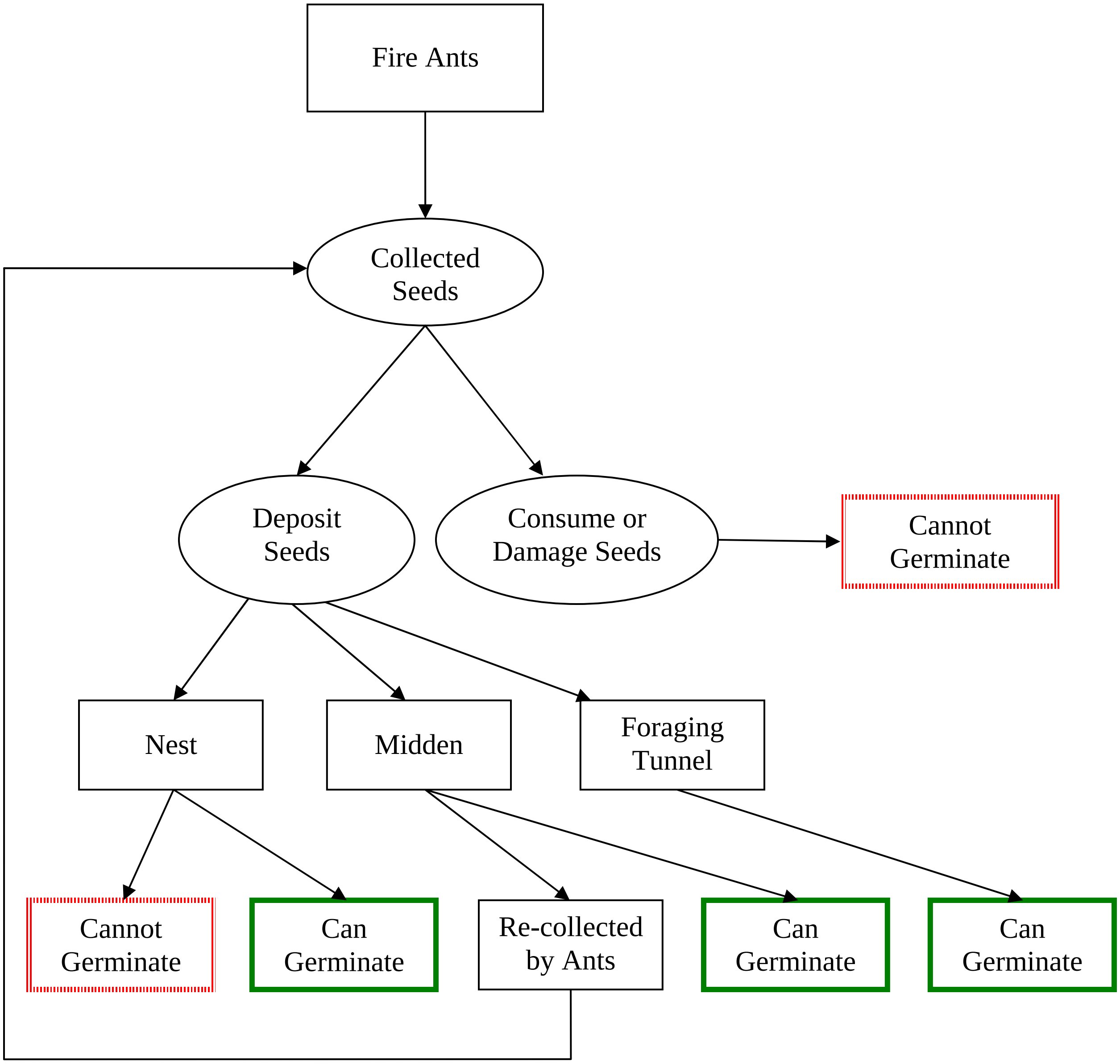

Figure 4.2 Seed fate once collected by fire ants. Circles indicate actions performed by the subjects which are indicated by boxes. The outcomes are shown with the bolder, green outlines indicating a possible germination ability of seeds and the dashed, red outlines indicating seeds are destroyed or cannot germinate.