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PASTURE MANAGEMENT, THE RED IMPORTED FIRE ANT (*SOLENOPSIS INVICTA*) AND DUNG BEETLE MEDIATED ECOSYSTEM SERVICES

by

CHRISTEN STEELE
B.S. Ohio University, 2007

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
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in the College of Sciences
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ABSTRACT

Grazing animals in Florida deposit over 20 million metric tons of dung per year, making dung a significant non-point source of pollution (extrapolated from Fincher, 1981). Degradation of this dung occurs naturally, primarily due to a diverse group of beetles (Order Coleoptera) primarily in the families Scarabaeidae and Geotrupidae (hereafter dung beetles). Dung that is not degraded becomes a non-point source of pollutants and may be leached into water bodies. Additionally, dung provides an incubation site for the pests and parasites of both humans and livestock. Thus, as dung beetles consume and degrade dung, they provide a multitude of ecosystem services by increasing the rate of dung decomposition in pasture ecosystems.

The non-native fire ant *Solenopsis invicta* has been observed to frequently utilize dung as a site to forage for the larvae of other insects. Based on the known food preferences of *S. invicta*, dung beetle adults and larvae fit the profile of a potential food source. Whether the ecosystem services provided by dung beetles are being reduced, un-impacted, or potentially increased through complementarity is unclear. Thus, this project sought to first map the distribution of *S. invicta* within pasture habitats along a disturbance gradient. Next, a field experiment was employed to test whether the interaction between *S. invicta* and native dung beetle communities impacts the provisioning of two ecosystem services: rate of dung degradation and parasite suppression.

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CHAPTER 1: INTRODUCTION

In Florida alone (a leading beef cattle producer), 1 million cattle graze nearly 5 million acres in the environmentally sensitive lands of the Greater Everglades Ecosystem (Anton, 2003). Subsequently, over 20 million metric tons of dung are deposited in Florida per year, making dung a significant non-point source of pollution (extrapolated from Fincher, 1981). Degradation of this dung typically occurs naturally, primarily as dung beetles bury and consume dung. However, livestock dung that is not degraded may lead to monetary losses to ranchers, nutrient leaching into the local waterways, and the spread of various pests and parasites that infect both livestock and humans (Nichols et al. 2008). As dung beetles degrade and reincorporate dung, they maintain nutrient cycles necessary for ecosystem function while also providing a multitude of direct services to land managers (Nichols et al., 2008). Thus, the optimization of the ecosystem services provided by dung beetles is a priority not only for the protection of terrestrial and aquatic biota, but also for the cattle industry. Understanding the biology, ecology and shifts in distribution, may aid in creating practices that promote the conservation of these economically and ecologically important organisms.

Dung Beetle Biology

Dung beetles are a wide ranging functional group comprised of many genera and species of beetles. They are found on every continent except Antarctica. However, their diversity is highest in tropical grasslands and savannas, where medium and large-sized mammals can often be found in high densities (Hanski and Cambefort, 1991). Adult dung beetles feed primarily by using soft mandibles made for sucking the liquid fraction of dung from the organic material.

Additionally, some species have gone through evolutionary transitions to alternative feeding modes, including granivory and predation (Simmons and Ridsell-Smith, 2011). Unlike the adult beetles, the larvae of dung-feeding beetles have mandibles capable of grinding the organic material within dung. Chemicals used as dewormers to rid livestock of gastrointestinal parasites, have been found to be toxic to the larvae of *Aphodius* dung beetles. Of the chemicals tested, ivermectin was found to have the highest toxicity with a lethal concentration of 0.88-0.98 mg of active ingredient per kilogram of dung dry weight (Hempel et al. 2006).

Reproduction in dung beetles can be divided into four primary reproductive strategies: 1) paracoprids (tunnelers) select fibrous portions of dung for rolling a brood ball. A single egg is placed within the center of the ball and it is then buried vertically beneath the dung pat; 2) telecoprids (rollers) also create fibrous brood balls, but they are moved a horizontal distance away from the pat before being buried beneath the soil; 3) endocoprids (dwellers) brood their young directly in the dung pat itself; 4) kleptocoprids only bury dung within brood balls created by other species of dung beetles (Nichols et al., 2008). These different strategies result in differences in the provisioning of ecosystem services. Endocoprids, for example, do not contribute to improvements to soil aeration and fertility as much as paracoprids or telecoprids because they do not create brood chambers and bury dung beneath the surface of the soil.

The high density of mammals within tropical systems creates a wealth of resources for dung beetles. A robust dung beetle community within the Amazonian basin can completely bury the droppings of most mammals within 48 hours after being deposited (Hanski and Cambefort, 1991). Although dung beetle species must compete for the same ephemeral resource, they are able to coexist due to high levels of temporal and spatial partitioning of the dung pat. For

example, dung beetles may exhibit differences in dung colonization time, seasonal activity and diel activity (Hanski and Cambefort, 1991; Chao, 2012). At the level of dung pat, dung beetle species also exhibit preferences in dung water content, odor and consistency. These preferences minimize direct competition for dung at the pat and allow for the community to work collectively to efficiently utilize the dung pat (Chao, 2012). Thus, the ecosystem services provided by dung beetle communities may be highly dependent upon the community assemblage and nesting strategies of the species found within a habitat type.

Within Florida, all four dung utilization reproductive strategies (rolling, tunneling, dwelling and kleptocoprids) have been found to be present. This includes two species of paracoprid beetles (*Onthophagus gazella* and *Euoniticellus intermedius*) introduced from South Africa to increase the rate of dung degradation. There have been few studies on the species distribution within the southeastern region of the U.S., but a recent study by Kaufman and Wood (2012) found over 39 species present in Alachua County, Florida (Table 1).

Table 1. List of Species found in Florida from Kaufman and Wood (2012). Beetles were trapped at four north central Florida cattle farms between June 2005 and December 2008 and are reported as the percent of total beetles captured.

Species	Beef 1	Beef 2	Dairy 1	Dairy 2
<i>Aphodius fimetarius</i> (L.) ^a	3 (0.03)	1 (0.01)	10 (0.03)	
<i>Ataenius cylindrus</i> Horn		12 (0.06)		
<i>A. erratus</i> Fall	35 (0.41)		1 (0.00)	
<i>A. imbricatus</i> (Melsheimer)	2 (0.02)			
<i>A. picinus</i> Harold	2 (0.02)			
<i>A. platensis</i> (Blanchard)	3 (0.03)	3 (0.02)	4 (0.01)	
<i>A. spretulus</i> (Haldeman)	2 (0.02)			
<i>Ateuchus lecontei</i> (Harold)	9 (0.10)	66 (0.33)	102 (0.31)	4 (0.26)
<i>Blackburneus aegyptus</i> (Horn)	3 (0.03)	13 (0.07)	65 (0.20)	
<i>B. rubeolus</i> (Beauvois)	346 (4.02)	115 (0.58)	813 (2.49)	16 (1.05)
<i>Calamosternus granarius</i> (L.) ^a	1 (0.01)	9 (0.05)	51 (0.16)	
<i>Canthon depressipennis</i> LeConte	1 (0.01)	51 (0.26)	141 (0.43)	
<i>C. pilularius</i> (L.)	29 (0.34)	128 (0.65)	443 (1.36)	9 (0.59)
<i>C. probus</i> (Germar)			1 (0.00)	
<i>C. vigilans</i> LeConte	1 (0.01)	5 (0.03)		
<i>C. v. viridix</i> (Beauvois)		3 (0.02)		
<i>Copris minutus</i> (Drury)	3 (0.03)	111 (0.56)	545 (1.67)	16 (1.05)
<i>Dichotomius carolinus</i> (L.)		4 (0.02)	4 (0.01)	1 (0.07)
<i>Euoniticellus intermedius</i> (Reiche) ^a	16 (0.19)	1,025 (5.18)	328 (1.01)	56 (3.67)
<i>Geotrupes egeriei</i> Germar	3 (0.03)	100 (0.50)	14 (0.04)	
<i>Irrasinus stupidus</i> (Horn)		16 (0.08)	214 (0.66)	
<i>Labarrus pseudolividus</i> Balthasar ^a	4,343 (50.44)	389 (1.96)	3,604 (11.06)	111 (7.28)
<i>Melanocanthon hispinatus</i> (Robinson)		2 (0.01)	4 (0.01)	
<i>Melanocanthon granulifer</i> (Schmidt)		2 (0.01)	20 (0.06)	1 (0.07)
<i>Mycotrupes gaigei</i> Olson and Hubbell			352 (1.08)	
<i>Onthophagus concinnus</i> Laporte		30 (0.15)	9 (0.03)	
<i>O. gazella</i> (F.) ^a	2,015 (23.40)	1,921 (9.70)	2,798 (8.58)	685 (44.95)
<i>O. hecate blatchleyi</i> Brown	725 (8.42)	421 (2.13)	212 (0.65)	8 (0.52)
<i>O. oklahomensis</i> Brown	4 (0.05)	168 (0.85)	282 (0.87)	1 (0.07)
<i>O. pennsylvanicus</i> Harold	207 (2.40)	1,279 (6.46)	53 (0.16)	
<i>O. taurus</i> (Schreber) ^a	792 (9.20)	344 (1.67)	2,294 (7.04)	568 (37.27)
<i>O. tuberculifrons</i> Harold	25 (0.29)	13,274 (67.03)	19,917 (61.11)	40 (2.62)
<i>Oscarinus crassulus</i> (Horn)		1 (0.01)		
<i>Os. windsori</i> (Cartright)			3 (0.01)	
<i>Parataenius simulator</i> (Harold)			5 (0.02)	
<i>Phanaeus igneus</i> MacLeay		138 (0.70)	248 (0.76)	
<i>P. vindex</i> MacLeay	37 (0.43)	162 (0.82)	57 (0.17)	8 (0.52)
<i>Pseudogolius bicolor</i> (Say)	1 (0.01)	9 (0.05)		
<i>Pseudocanthon perplexus</i> (LeConte)	3 (0.03)			
Total	8,611	19,591	32,594	1,524

N = 10 traps per farm. Dairy 2 sampled 16 May to 18 December 2007.

^a Introduced species.

Ecosystem Services Provided by Dung Beetles

Soil and Vegetation Services

Due in part to the warm climate of southeastern U.S., without dung beetle activity, weathered dung will lose up to 80% of its nitrogen to volatilization (Gillard, 1967). Mesocosm experiments have found that when dung is incorporated into the soil by dung beetles, soil fertility

and plant growth are significantly enhanced (Yokoyama et al., 1991; Bang et al., 2005). Bang et al. (2005) conducted experiments with 3 species of dung beetles, both in the lab and the field, in an effort to quantify the impact of beetle activity on pasture herbage. Despite the small number of species that were used, the authors found that the presence of the beetles increased air permeability of the soil up to 10cm and increased digestibility and total crude protein within grass shoots relative to the control. Overall, dung beetle activity increased the value of pasture herbage through both the incorporation of dung into the soil and through soil aeration.

In total, it is estimated that dung beetle mediated fertilization provides 691 million kg of nitrogen to U.S. pasture soils per year, thereby reducing the need for costly chemical fertilizer (Losey and Vaughn, 2006). Furthermore, dung beetle activity has also been found to increase soil micronutrients (Ca, Mg, K) and soil pH (Bertone, 2004; Lastro, 2006; Yamada et al., 2007). This aids in further reducing the need for land managers to purchase costly fertilizing inputs such as lime and mineral for pastures. The benefits provided by dung beetles are therefore of great interest to ranchers as they contribute directly to economic gains while also maintaining the functioning of the grass-based agro-ecosystem.

Vertebrate Health Services

Dung incorporation into the soil by dung beetles kills many pests and parasites that reproduce within dung pats (Miller, 1961; Fincher, 1973). Contamination of pastures with the larvae of such parasites may lead to health threats to wildlife, humans and livestock. Gastrointestinal parasites are common within livestock and may cause weight loss, wasting, and even death. As adults, gastrointestinal parasites release eggs into the feces of the infected animal.

These eggs then hatch into larvae and by their 3rd molt, the larvae are capable of moving out of the dung pat and into the surrounding herbage where they can be consumed by grazing mammals (Fincher, 1973). Miller (1961) found that because the adult mouthparts of the dung beetle function to simply filter (not macerate) dung, the eggs and larvae of livestock parasites are often passively damaged during the filtration process. As an additional suppressant, the burial of dung by beetles is thought to also prevent parasites from finding the necessary microhabitat to complete their life cycle. For example, Bryan (1976) found that control dung pats without the dung beetle *Onthophagus gazella*, had 50 times more livestock parasitic nematode larvae than pats with 10- 30 beetle pairs. Thus, dung beetle regulation of parasites and protozoa are part of a process whereby populations are reduced as the beetles feed and reproduce within the dung.

In addition to various parasites regulated by dung beetle activity, a variety of dung breeding flies are also reduced. Dung breeding flies, such as *Haematobia irritans*, bite through the hide of livestock to obtain a blood meal. These insects can cause great stress to livestock and may significantly reduce productivity and hide quality when densities are high (Fincher, 1981). Experimental manipulations both in lab and field studies have shown that dung beetle activity increases fly mortality through a variety of means, including direct competition for dung resources, damage of eggs via beetle mandibles, and disruption of the dung microclimate through aeration of dung pat (Simmons and Ridsell-Smith, 2011; Bishop et al., 2005; Ridsell-Smith and Hayles, 1987).

Potential Threats to Dung Beetle Communities

Habitat Destruction

Dung beetle community response to habitat disturbance has been studied primarily in tropical ecosystems. Slade et al. (2011) surveyed beetle diversity within undisturbed tropical forest, selectively logged forest and high-intensity logged forest. The researchers found the lowest species richness at the high-intensity site, but biomass and abundance did not show the same pattern. This was due primarily to density compensation; as species were extirpated at the high-intensity site, competition was reduced thereby providing less sensitive species an opportunity to preferentially utilize available resources. The research team also investigated how such changes in species composition within forests of differing logging intensity impacted two ecosystem services provided by dung beetles: dung degradation and seed removal. A strong positive correlation between species richness and dung removal was found. This suggested that the relationship was rather robust and even a small increase in richness resulted in an increase in the service of degradation. Although the high-intensity logged sites had greater abundance of beetles, the loss of complementarity between species was of greater impact to ecosystem service provisioning in this system.

Although few studies of the relationship between habitat change and dung beetle diversity have occurred in agro-ecosystems, a study by Hutton and Giller (2003) showed a negative trend similar to that found within tropical systems. In a dung beetle survey across organic, rough, and intensive sheep farms in Ireland, the abundance, biomass, richness and diversity of beetles was found to be significantly higher on organic sites. The authors hypothesized that this was likely due to the reduction in the heterogeneity of farmland brought

about by the intensification of production. Intensive farm types lacked the hedgerows and native vegetation present within organic sites and these features have already been attributed to increased bird, centipede and carabid beetle diversity at organic sites within the region (Hutton and Giller, 2003). However, because parasiticides were sometimes found within the dung pats, due to worming of the animals at intensive sites, the results may be confounded by the impact of dung toxicity on dung beetle survival.

Invasive Species

In addition to dung beetles, various ant species occasionally forage within dung pats. Within the southeastern U.S., the invasive red imported fire ant, *Solenopsis invicta*, has been found to be a frequent visitor of dung pats as it exploits the larvae of dung breeding flies as a food source (Summerlin et al., 1984a). This selective foraging behavior significantly reduced the abundance of *Haematobia irritans*, the horn fly, relative to ant-free control pats within a study in the US (Summerlin et al., 1984a).

If or how *S. invicta* might be interacting with dung beetles within pasture systems of the southeastern United States remains unclear. Summerlin et al. (1984b) obtained observational data on the interactions between dung beetles and *S. invicta* and determined that while beetles were not discouraged from inhabiting pats by the presence of the ants, fewer adults appeared to emerge from the pats (Summerlin et al., 1984b). However, the vegetation and soil disturbance which commonly occurs with agricultural intensification may increase the invasibility of pastures to *S. invicta* (Wilson, 1951; King and Tschinkel, 2008; Stuble et al., 2009). Because *S. invicta* utilize dung as a food resource, it is possible that they are competitively excluding dung

beetles from dung pats, or actively depredating them within disturbed sites (Hu, 1996). Either interaction would have deleterious effects on the ecosystem services dung beetles provide and may increase the abundance of pests and parasites within dung pats (Coppler, 2007). Further study is necessary to determine how this and other dung inhabiting insects may interact with dung beetles to impact the delivery of ecosystem services.

Proposed Research

Despite their ecological and economic significance, little is known about how management techniques within rangelands influence dung beetle communities and their ecosystem services. My proposed research will utilize both observational and experimental studies to determine how habitat variables altered by pasture management influence dung beetle communities, *S. invicta* densities and how interactions between the two groups of organisms might impact the ecosystem services provided by dung beetles. For the observational study, I will sample dung beetle communities and survey *S. invicta* densities across pastures of varying management intensity. I will then experimentally manipulate the access of beetles and ants to dung pats to evaluate how beetle community composition and the presence or absence of *S. invicta* influence the ecosystem services of parasite suppression and dung degradation.

CHAPTER 2: VARIATION OF FIRE ANT DENSITIES ACROSS ENVIRONMENTAL GRADIENTS IN CENTRAL FLORIDA PASTURES

Introduction

Between 1933 and 1945, the invasion of the southeastern US by *Solenopsis invicta* Buren (Hymenoptera: Formicidae: Myrmicinae), or the red imported fire ant, began accidentally with introductions to Mobile, Alabama from soil used in the ballast of ships arriving from Paraguay. The species soon colonized soil within greenhouses and plant nurseries in the Mobile area and this led to dispersal through the shipments of nursery stock to cities throughout the southeast. This human-aided dispersal was abetted by the ability of *S. invicta* to form whole colony rafts and disperse along waterways during flood events (Tschinkel 2006). Today, *S. invicta* inhabits every state within the southeastern U.S. and has also been introduced from the U.S. population to Australia, southern China, Taiwan and Hong Kong (Ascunce et al. 2011) (Figure 1).

Despite over 70 years of residency in the U.S., the ability of *S. invicta* to invade new habitats and persist within them is a contentious topic of study that has yet to be fully understood. Certain abiotic factors such as soil temperature (Porter and Tschinkel 1993) and soil moisture (Xu et al. 2009) have been found to impact the rate of growth of *S. invicta* colonies within its invaded range. However the importance of these variables to the density of mounds at the habitat-scale is unclear. Furthermore, various forms of anthropogenic habitat disturbance have been shown to both have no impact (Camilo and Phillips Jr. 1990, Porter and Savignano 1990, Morris and Steigman 1993) and positively correlate with *S. invicta* densities (Stiles and Jones 1998, Forsy et al. 2002, King and Tschinkel 2008, LeBrun et al. 2012). Thus, a better understanding of the role of anthropogenic disturbance and other habitat-scale variables that may

control *S. invicta* density is necessary, and may provide greater insight into the species persistence or potential management solutions.

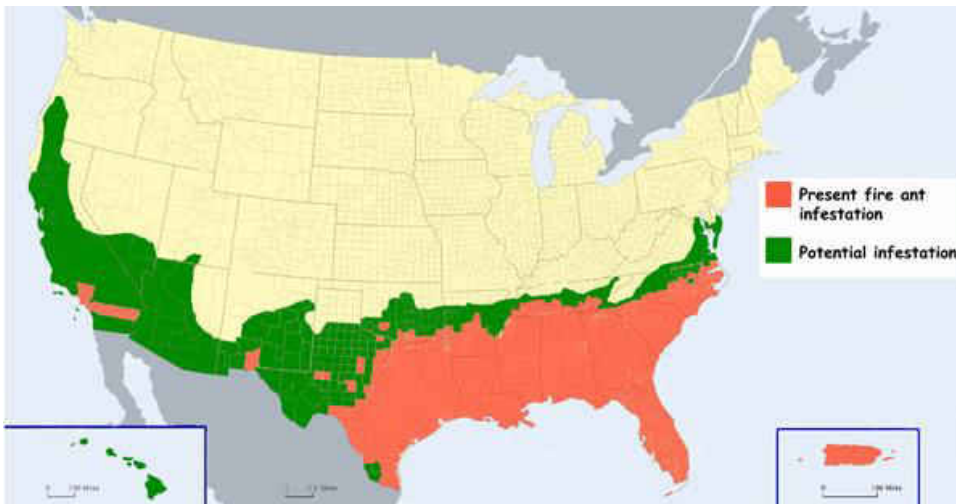


Figure 1: Map produced by the United States Department of Agriculture, Agricultural Research Service depicting the current known range of *Solenopsis invicta* in pink and the potential range depicted in green.

The Role of Abiotic Variables

The growth of a colony is highly dependent upon the ability of worker ants to successfully forage for resources. Soil temperature influences the speed at which worker ants are able to move through foraging tunnels to acquire food; with foraging activity maximized between 22 and 36°C (Porter and Tschinkel 1993). This preference for soil temperature creates both a differences in foraging rates between seasons and in habitats with or without shade (Porter and Tschinkel (1987). Survival of workers is also dependent upon soil moisture, with values of 0.25 mean water content within the nest resulting in 45% mortality of acclimated workers (Xu et al. 2009). Although the study only measured moisture tolerance of the ants up to 35% relative soil moisture, no negative impacts of high moisture were noted, indicating that soil aridity may

be a limiting factor for the species. Combined, seasonal changes in soil temperature and soil moisture maintain an annual cycle of colony growth and reproduction, however their impact on the density of *S. invicta* colonies surviving within a habitat is unclear (Tschinkel 2006).

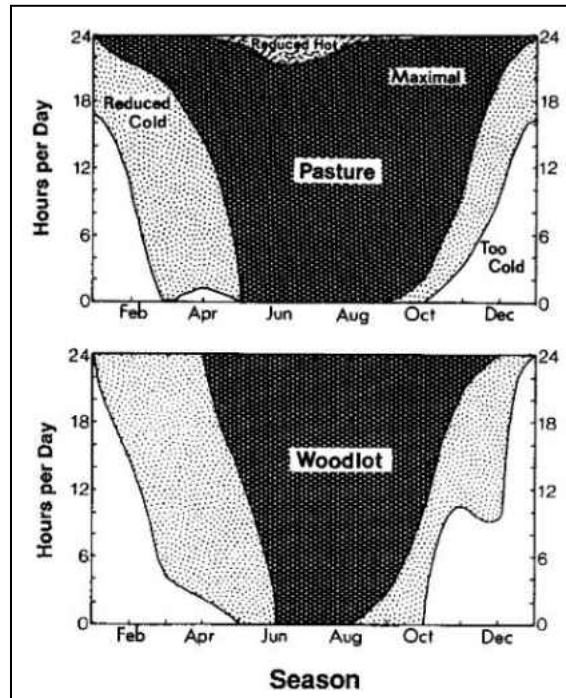


Figure 2: Redrawn from Tschinkels' (2006) *The Fire Ants*, showing how hours of sunlight per day and season affect foraging rate in pastures (top) and woodlots (bottom).

The Role of Edge Habitat

Perhaps due in part to the range of soil temperature and moisture conditions tolerated by *S. invicta*, edge habitats created by roads and development have been demonstrated to house high densities of the species (Stiles and Jones 1998, Forsys et al. 2002). In the Florida Keys in the late 80's, the density of *S. invicta* was relatively low, as invasion had not yet occurred on every island (Porter 1992). By 2002 a study by Forsys and Allen found high densities along roadways and in edge habitats in every Key. Furthermore, undisturbed sites were more likely to be occupied if

they were within 50m of a road and within 150m of high densities of urban development. This indicates that the conditions within edge habitats allow for persistence and eventual dispersal into undisturbed sites (Forys, Allen et al. 2002). However, the specific conditions that appeal to *S. invicta* within edge habitats have yet to be fully quantified (Tschinkel 2006, King and Tschinkel 2008).

The Role of Grazing

Pasture environments have been found to be occupied by *S. invicta* in some of the highest densities of any habitat type, besides roadsides, making them an excellent location to investigate the distribution of the species along abiotic gradients (Tschinkel 2006). Within pastures, a common form of disturbance arises from the grazing activity of livestock. Tucker et al. 2010 found that within the same pasture, there were more *S. invicta* workers at baits where grazing was occurring compared to areas excluded from grazing. However, the mechanism by which grazing might influence density is not clearly understood. Research by Bremer et al. 2001, found that evapotranspiration within pasture soils decreased in response to grazing, thereby increasing soil moisture (Figure 3). Thus, due to research demonstrating the species preference for moist soils, grazed pastures may better maintain soil moisture levels suitable for foraging than ungrazed pastures. (Xu, Zeng et al. 2009). Overall however, the ability of grazing to alter the pasture landscape is largely dependent upon the management methods utilized by ranchers and the disturbance history of the pasture.

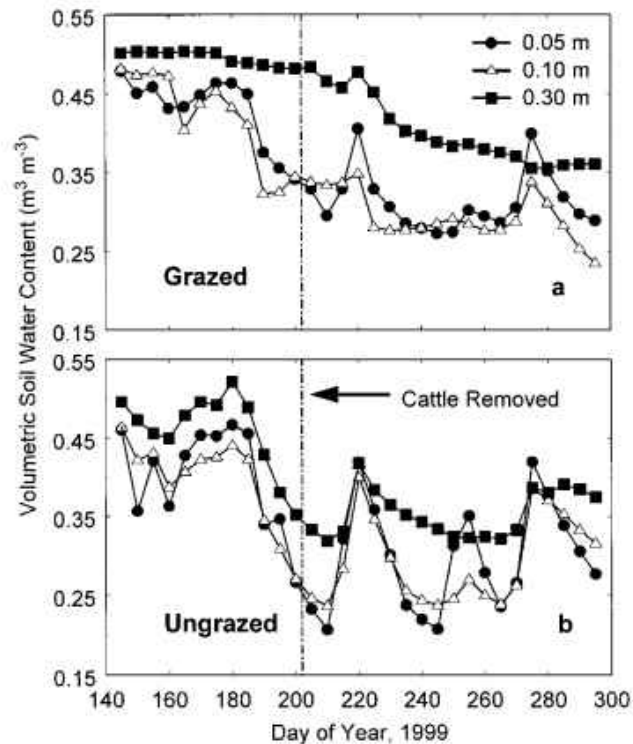


Figure 3: Bremer et al. 2001 found that grazing reduced evapotranspiration by 28%, which resulted in a conservation of volumetric water content (θ_v) in grazed prairie sites.

The Role of Historical Habitat Disturbance

Studies from various locations throughout the invaded southeast have described conflicting stories about the ability of *S. invicta* to invade, and therefore persist in, historically undisturbed, or pristine, habitat. In Texas, Morris and Steigman (1993) found that *S. invicta* was invading both disturbed and virgin parcels of blackland prairie with equally high densities of 200-300 mounds per hectare. Similarly, declines in native ant species were observed as both disturbed and undisturbed sites were invaded in central Texas (Camilo and Phillips Jr. 1990). Such a ubiquitous pattern of invasion would suggest that the ants are omnivorous, habitat

generalists capable of invading most habitats where the required abiotic and biotic conditions are met and that sites with or without habitat disturbance are equally suitable.

Conversely, recent experimental studies on the occurrence of *S. invicta* in natural habitats have focused on historical habitat disturbance as a precursor to invasion. An experimental study by King et al. 2008 was conducted in undisturbed and uninvaded pine flatwoods in Florida. Plots were mowed, plowed or unaltered and then received either an addition of mature *S. invicta* colonies or no ant addition. While *S. invicta* persisted in all plots where colonies were added, survival and growth were highest in plots disturbed through mowing or plowing. Furthermore, *S. invicta* colonies were found to naturally recruit into plowed plots in high densities, whereas undisturbed plots received no natural recruitment. This suggests that, within this system, the invasive nature of *S. invicta* is dependent upon both dispersal ability and the presence of habitat conditions consistent with disturbance. Similarly in southeast Texas, LeBrun et al. (2012) compared the prevalence of *S. invicta* in undisturbed and disturbed sites and found that while the ants were present in undisturbed habitats; their prevalence was significantly higher in disturbed sites. The disturbance utilized for this study occurred anywhere from 5-35 years prior to the study and as time since disturbance increased, *S. invicta* densities were found to return to the low levels observed in undisturbed habitats.

The goal of this study was to understand if abiotic variables, distance to edge habitat and a history of anthropogenic disturbance influence the density of *S. invicta* within pasture habitat. To accomplish this, I measured the dependent variable, *S. invicta* mound density, across a range of environmental conditions on a south-central Florida cattle ranch. Explanatory variables were

grouped into 5 categories in order to develop a regression model that would best predict the density of *S. invicta*:

- (1) historic pasture management (conversion of native habitat to semi-native or intensive pasture)
- (2) modern pasture management (mowing, dragging, chopping or aerating)
- (3) grazing intensity (average vegetation height and number of cow dung pats)
- (4) distance to human-made and natural edge habitat (roads, ditches and wetlands)
- (5) abiotic conditions (soil temperature, soil moisture)

The primary hypotheses were that if habitat disturbance is the primary driver of *S. invicta* habitat selection, then density will be correlated with variables related to the degree of disturbance and the exposure of soil.

Methods

Study Site

The study was conducted at MacArthur Agro-Ecology Research Center at Buck Island Ranch (hereafter MAERC) a 4,170-ha commercial cattle ranch, with approximately 3,000 cow-calf pairs located in Lake Placid, Florida. MAERC is part of Archbold Biological Research Station (Figure 4).

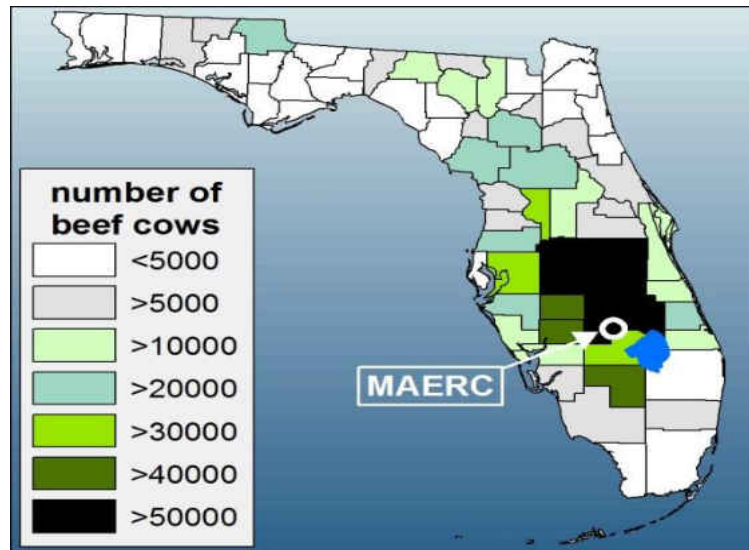


Figure 4: The study site, MAERC, is located in an area of the highest density of cattle ranching in Florida (Ewing, 2013).

The subtropical climate has distinct wet and Dry Season seasons and an average annual rainfall of ~130 cm. Most soils at MAERC are poorly drained, acidic, sandy spodosols, alfisols and entisols. MAERC is divided approximately 50:50 into two pasture types: intensively managed and semi-natural (Figure 5). Intensively managed pastures are planted with introduced Bahia grass (*Paspalum notatum*) and have been fertilized annually with NPK fertilizer for 20+ years prior to 1987. They have been fertilized only with N annually since 1987. In contrast, semi-natural pastures are not known to have ever been fertilized and are dominated by native grasses, such as bunchgrasses, (e.g., *Andropogon virginicus* and *Panicum* spp., *Axonopus* spp.). *Solenopsis invicta* colonies are commonly found throughout both pasture types.



Figure 5: Image showing the marked differences in the vegetation found in the semi-native pastures (on left) and intensively managed pastures found at MAERC.

Sampling

From the 34 pastures available in the northern section of the Macarthur Agroecology Research Center, 8 pastures were selected (4 each in semi-native and intensively managed pastures) to conduct *S. invicta* surveys. Because *S. invicta* density has been found to increase with increasing human disturbance, plots within pastures were placed using stratified random sampling by disturbance (i.e. proximity to road) (King and Tschinkel 2008). Approximately 1/3 of the plots were placed in disturbed areas predicted to house a high density of fire ants and the remainder of the sites were randomly located within the pasture. Additionally, the number of plots within a pasture were based upon pasture size (1 per 100 hectares).

In total, 56 plots were surveyed twice across the eight pastures (Figure 6). Each plot was 26×26 m in size and was searched entirely for fire ant mounds. Surveys consisted of walking 13, 2 m wide transects across each plot while scanning for mounds. Surveys were first conducted in

January of 2014 and then repeated in May of 2014. During each sampling event abiotic variables, vegetation height and number of cow pies (dung piles) within a 2x2 subplot at the center of the plot, were recorded (Appendix). The distance of each plot to the nearest road, wetland and ditch was then determined using shapefiles created by Archbold Biological Station with ArcMap 10.2.2.

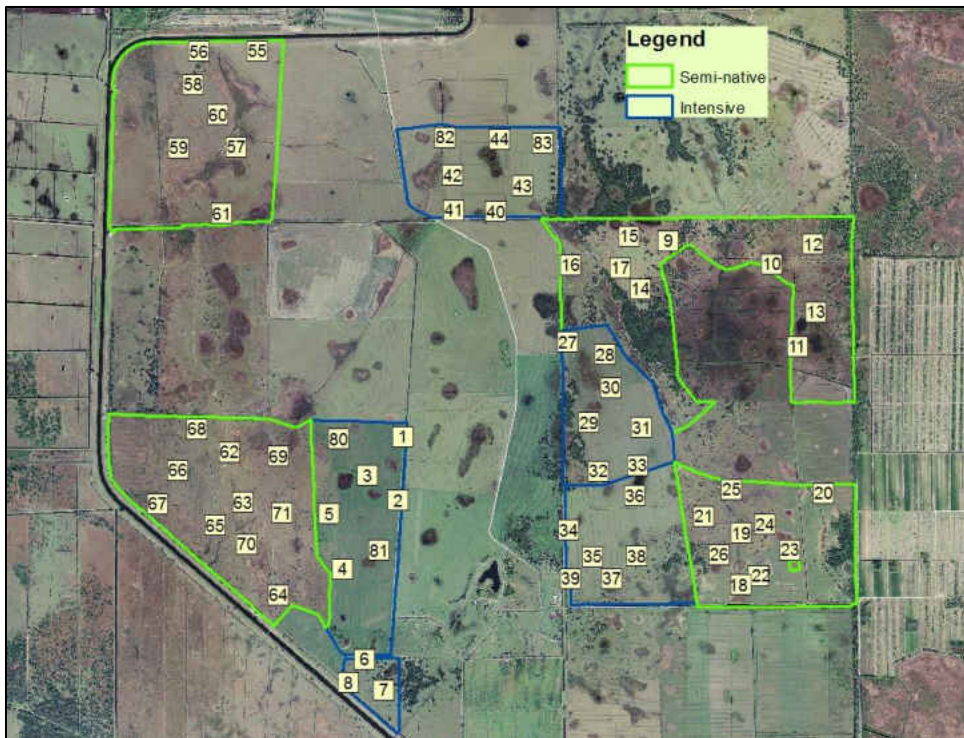


Figure 6: Map depicting the location of plots within semi-native (green outline) and intensively managed (blue outline) pastures.

The management history for each pasture was also collected from a database developed at MAERC known as PastureSTAR. The date and percentage of pasture covered by the management activity are recorded in PastureSTAR for the following activities: mowing, dragging, roller chopping and aerating. Mowing of pastures usually involves cutting vegetation to 15cm in height or shorter in order to promote the growth of grass and reduce the growth of

forbs and woody vegetation. Dragging involves utilizing a tractor to pull weights (often tractor tires) through the pasture in order to physically break apart dung pats on the surface of the pasture. This too is done in order to promote the growth of forage grasses. Roller chopping and aerating are similar in that they both utilize a tractor attachment designed to disturb the surface of the soil (Figure 7). An aerator attachment is a large roller with spikes that is used to puncture the surface of the soil. A roller chopping attachment is also a large roller, but with blades instead of spikes that cut vegetation near the surface of the soil. Roller chopping also disturbs the first 5-15 cm of soil. Although soil disturbance has been found in several studies to be an important habitat variable that promotes *S. invicta* establishment (Stiles and Jones 1998, King and Tschinkel 2008, LeBrun, Plowes et al. 2012), the less invasive activities of mowing and dragging may also increase habitat suitability (King and Tschinkel 2008).



Figure 7: Aeration of pastures (left) is accomplished by punching holes into the first 5-15 cm of soil. Similarly, chopping of pastures (right) is designed to cut down woody vegetation and tends to disturb the first 5-15 cm of soil.

Analysis

The response variable analyzed was the total number of active mounds per plot. The data collected for this study were nested at 2 different levels. The primary units were the pastures, which were randomly selected from the larger pasture type blocks. Randomly located plots were

then sampled within each pasture. Due to the hierarchical design of the study and the count response variable, generalized linear mixed models were utilized with 'Pasture' assigned as a random factor. Dry season (January) and wet season (May) surveys were analyzed separately due to previous evidence that fire ant densities vary with season and because of differences in the abiotic variables collected in each season (Porter and Tschinkel 1987, Porter 1988).

Pasture management history predictors were analyzed individually as well as grouped into two categories: 1) Ground surface disturbance (mowing and dragging) and 2) Soil disturbance (aeration and chopping). All pasture history variables were denoted as the amount of time from 2014 until the last management activity. For example, mowing a pasture in 2007 was recorded as 7 years since activity, while mowing in 2013 was recorded as 1 years since activity.

Overall, *a priori* hypotheses were used to group the 10 explanatory variables into 4 categories. These included: 1) historic pasture management (semi-native or intensively managed), 2) modern pasture management (mowing, dragging, chopping or aerating, 3) grazing intensity (average vegetation height and number of cow dung pats, 4) distance to human-made and natural habitat features (roads, ditches and wetlands, 5) abiotic conditions (soil temperature for the Dry Season data, soil moisture for the Wet Season data). Collinearity among predictor variables was assessed and no correlation coefficients were greater than 0.75, therefore no variables were omitted.

Due to the large number of variables and the large number of potential models that could be created, I followed a procedure adopted from the collective works of Zuur to reduce the model set (Zuur et al. 2007, Zuur et al. 2009, Zuur et al. 2010). After constructing the 4 hypothesis categories, ten possible two-way interactions were added based on *a priori* biological

knowledge. Within each hypotheses category, models were constructed by means of maximum likelihood (ML) and the lowest AICc was used to select the best-fit model (Burnham and Anderson, 2002). The variables within the top models from each category were then used to construct a final set of models that were again compared using AICc. After examining the models for each hypotheses group, only 4 variables within the Wet Season data set produced models with AIC values lower than the null, while 6 were variables were found for the Dry Season data set. These variables were then combined across hypotheses groups to produce the final model set. This process generated 15 possible models for the Wet Season data and 32 for the Dry Season data. Graphs were used to visually check for homogeneity and normality of data.

Results

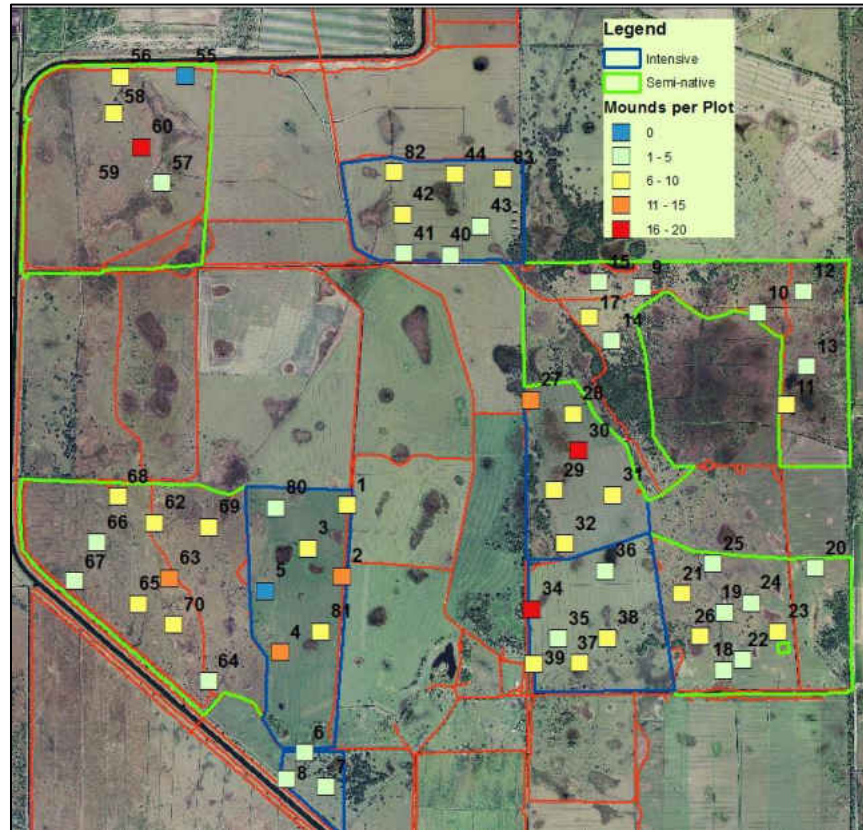


Figure 8: Map of the densities of *Solenopsis invicta* within each plot sampled during the Dry Season sampling event.

Of the 57 plots sampled in the Wet Season and Dry Season, only 2 were found to be naturally devoid of *S. invicta* mounds (Figure 8). The average number of mounds per plot was higher in the wet season than in the Dry Season season in both pasture types and higher in the intensive plots than in the semi-native plots (Table 2).

Table 2: Descriptive statistics for the number of mounds per plot found in each pasture type during the wet and dry season.

		Dry Season	Wet Season
	Mean	5.84	6.64
Intensive	95% CI Upper	4.12	4.66
	95% CI Lower	7.56	8.61
	Std. Deviation	4.16	4.78
	Maximum	16	18
Semi-native	Mean	3.65	5.00
	95% CI Upper	2.68	3.46
	95% CI Lower	4.61	6.54
	Std. Deviation	2.64	4.19
	Maximum	12	19

In both wet season and dry season, the amount of time passed since soil disturbance (aeration and chopping) was the most significant predictor of *S. invicta* densities, with greater soil disturbance being associated with higher mound densities (Figure 9). Other forms of pasture management that did not disturb the soil, such as dragging and mowing, were not found to be significant in either season. Seasonally, the interaction between pasture type and vegetation (Type:Veg) was significant within the dry season model. For the wet season data, the number of dung pats per plot (Pies) was significant, with more dung pats associated with a greater number of ant mounds. The results by season are discussed in greater detail below.

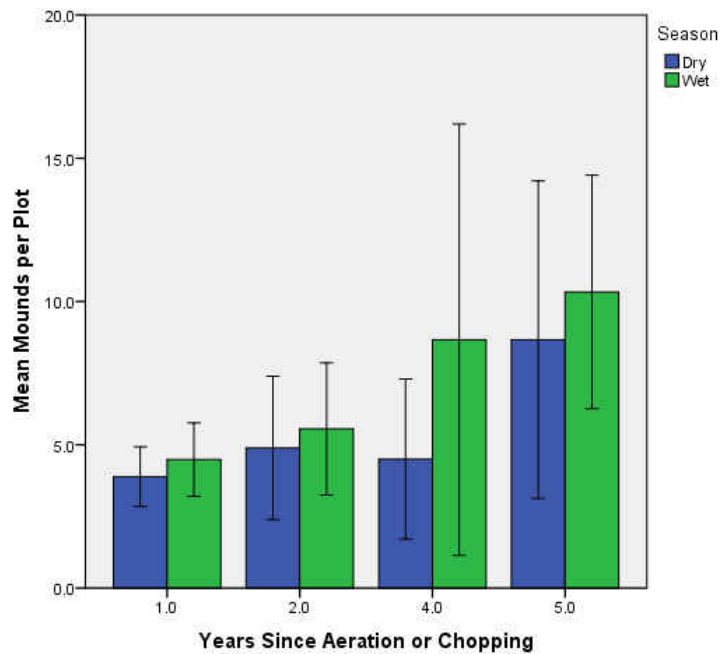


Figure 9: Graph of the number of mounds in plots that had been aerated or chopped 1, 2, 4 and 5 years prior to the sampling event. No plots were aerated or chopped in 2012, resulting in no results for 3 years since disturbance. Error bars were constructed using a 95% confidence interval of the mean.

Dry Season Data

For the dry season data set, the following variables were within the top models from each hypothesis category and were used to construct the final set of models: number of cow pies (Pies), vegetation height (Veg), time since aeration or chopping of pasture (TSA_C), time since mowing or dragging of pasture (TSM_D), pasture type (Type) and an interaction between the pasture type and the height of vegetation (Type:Veg). Notably, none of the models containing distance to edge habitat variables were found to possess more predictive power than the null model. Of the 32 possible models created for the dry season data set with these variables, the top 7 were nearly indiscriminate in their delta AICc values and weights (Table 3). However, all 7 included the variables TSA_C + Type:Veg. These variables were therefore selected as the most parsimonious model since they were contained as subsets within all top models. Both variables

were found to be significant within the model (Table 4). Time since aeration/chopping was positively related to mound density, suggesting that there were fewer mounds in more recently disturbed pastures. There was a significant interaction between pasture type and vegetation (Type:Veg) suggesting that there was no effect of vegetation height on mound abundance in improved pastures, while in semi-native pastures, # of mounds was negatively related to vegetation height.

Table 3: All models for the **dry season** data with a difference between the lowest AICc value and AICc_i of 2 or less. The following variables were used for model construction: ‘Pies’ (number of dung pats per plot), ‘TSA_C’ (time since soil disturbance/aeration or chopping of pasture), ‘Veg’ (average vegetation height within each plot), ‘TSM_D’ (time since mowing and dragging of pasture) and ‘Type:Veg’ (an interaction between the pasture type and average vegetation height per plot). Log(L) = maximized log-likelihood, K= number of parameters, AICc = Akaike information criterion value, ΔAICc = differences between the lowest AICc value and AICc_i, AICcWt = model weight.

Model	Log(L)	AICc	ΔAICc	df	Weight
TSA_C + Type:Veg + Pies + Veg	-132.7	279.2	0	6	0.1163
TSA_C + Type:Veg	-131.5	279.4	0.2	7	0.1077
TSA_C + Type:Veg + Pies	-134.1	279.4	0.2	5	0.1041
TSA_C + Type:Veg + Veg	-133.1	279.9	0.6	6	0.0842
TSA_C + Type:Veg + TSM_D	-132	280.4	1.2	7	0.0633
TSA_C + Type:Veg + Veg	-133.4	280.5	1.3	6	0.0614
Global	-131	281.1	1.9	8	0.0444
TSA_C + Type:Veg + TSM_D + Veg	-132.5	281.3	2.1	7	0.0402

Table 4: Summary of results from the top model for the Dry Season data set: Type:Veg + TSA_C.

	Estimate	Standard Error	Z value	Pr(> z)
(Intercept)	1.49077	0.17669	8.44	<2e-16
TSA_C	0.15149	0.05862	2.58	0.0098
Type:Veg	-0.00879	0.00291	-3.02	0.0025

Wet Season Data

For the wet season data set, the following variables were within the top models from each hypothesis category and were used to construct the final set of models: number of cow pies (Pies), time since aeration or chopping of pasture (TSA_C), pasture type (Type) and an interaction between the time since aeration or chopping and pasture type (TSA_C:Type) (Appendix A). Similarly to the dry season data, none of distance to edge habitat were found to possess more predictive power than the null model. Of the 15 possible models created with these variables, the model that included Pies and TSA_C was selected as the top model as it had a weight nearly triple that of the next most plausible model (Table 5). Both variables were found to be significant within the model (Table 6). The average number of mounds per plot increased as both the time since aeration or chopping increased and as the number of pies increased.

Table 5: All models for the **wet season** data with a difference between the lowest AICc value and AICc_i of 2 or less. The following variables were used for model construction: ‘Pies’ = number of dung pats per pasture, TSA_C = Time since chopping and aeration of pasture, and Type = Pasture type (semi-native or intensive). Log(L) = maximized log-likelihood, AICc = Akaike information criterion value, ΔAICc = differences between the lowest AICc value and AICc_i, AICcWt = model weight.

Model	Log(L)	AICc	ΔAICc	df	Weight
Pies + TSA_C	-144.7	300.7	0	5	0.242
Type + Pies + TSA_C	-144.4	302.6	1.9	6	0.091
TSA_C	-147	302.7	2	4	0.087

Table 6: Summary of results from the top models for the Wet Season data set: Pies + TSA_C.

	Estimate	Standard Error	Z value	Pr(> z)
(Intercept)	0.9146	0.2466	3.71	0.00021
Pies	0.0515	0.0246	2.09	0.03632
TSA_C	0.2146	0.0693	3.1	0.00195

Discussion

Studies conducted on the distribution of *S. invicta* at regional scales have found abiotic variables, such as soil temperature, moisture and climate, to play a significant role in both presence and density in a habitat (Tschinkel 2006). However, less is understood about how these variables and others may influence the distribution of the species at the habitat scale. In this study, pasture habitats within south Florida were utilized to investigate how *S. invicta* density is influenced by abiotic variables (temperature and moisture), distance to edge habitat, pasture management history (semi-native vs. intensively managed pastures) and time since management activity (chopping, aerating, mowing and dragging). We expected *S. invicta* density to be positively correlated with distance to edge habitat, time since management activity and more prevalent in intensive pastures. Yet, the only management activity to significantly predict *S. invicta* density was time since chopping or aeration. The number of cow pies within a pasture and the vegetation height within each pasture type were also found to predict density in the wet and dry season, respectively.

Time since Soil Disturbance (Aeration or Chopping)

Our study found that time since soil disturbance was a driving variable for both dry season and wet season fire ant mound density. Mound densities were lowest in sites with less time since disturbance and increased as time since soil disturbance increased from 1-5 years. A study by LeBrun et al., the prevalence of *S. invicta* was assessed in Texas pastures from 5 to 35 years after an initial soil disturbance. They found an overall increase in prevalence after 5 years, followed by a gradual decline as time since disturbance increased to 35 years. While the results

from this study support the work of LeBrun et al. with an overall increase in *S. invicta* density at 5 years post-disturbance, a decline in density was also observed in the first year after disturbance (Figure 5). After 4-5 years, densities were found to rebound to levels above the average density per pasture.

The initial disturbance of the uppermost layers of soil associated with pasture management activities likely destroys many of the existing mature colonies of *S. invicta*, a species that is dependent upon building an aboveground mound structure. After the disturbance has occurred, newly established *S. invicta* colonies are able to grow exponentially and within 5-6 years a single colony will have up to 200,000 workers (Tschinkel 1988). The ants proclivity for rapid growth coupled with a removal or reduction of native ant colonies due to soil disturbance, may then lead to an expeditious rise in the number of *S. invicta* colonies within the disturbed habitat (King and Tschinkel 2008, Tschinkel 2006).

Vegetation Height and Pies: One and the same?

In agricultural studies, vegetation height is often used as a measure of grazing intensity due to the patchiness of livestock grazing activity. However, in semi-native pastures where species composition (and therefore vegetation height) may vary greatly within and between pastures, another method of estimating grazing intensity may be needed. Thus, for this study we counted the number of dung pats found within a 4m² area of the plot as a proxy for grazing intensity. Surprisingly, there was only a weak correlation between ‘Pies’ and ‘Veg’ within the wet season data set ($r=-0.3868$). Additionally, the number of dung pats within a plot proved to be a significant variable for the wet season data, while vegetation height did not. This may be

because dung pats are not only an indicator of grazing intensity, but also provide an additional benefit to *S. invicta* in the form of moisture refugia and a foraging site.

During sampling, it was observed that many of the dung pats within plots were home to small colonies of *S. invicta*. It is possible that even in mesic pastures, dung pats provide shelter for colonies as the hardened crust of the pat reduces water loss from the inner portions of dung. Furthermore, pats are home to the larvae of numerous species of dung breeding flies as well as adult and larval dung beetles. The concentration of these organisms in the small area of a dung pat likely provides an easily foraged source of high protein. Further investigation of the relationship between *S. invicta* and dung pat usage is necessary to determine how colonies and foraging workers may be utilizing dung pats in pastures to maintain high densities.

Interaction between Type and Vegetation

Only the Dry Season data found a significant interaction between pasture type (Type) and vegetation height (Veg) within plots. As can be seen in Figure 10, within semi-native pastures the vegetation height is highly linear, with the number of mounds declining sharply as vegetation height increases. However, in the intensive pastures the relationship between mound density and vegetation height is much more variable and no firm relationship emerges. This may be related to grazing intensity within the intensive pastures. Lower vegetation height would be indicative of higher grazing intensity and perhaps greater abundance of Bahia forage grass. As shown in the work of Bremer et al. 2011, greater grazing intensity may aid in retaining soil moisture. Thus, higher grazing intensity in the intensive pasture may have resulted in higher densities of *S. invicta*.

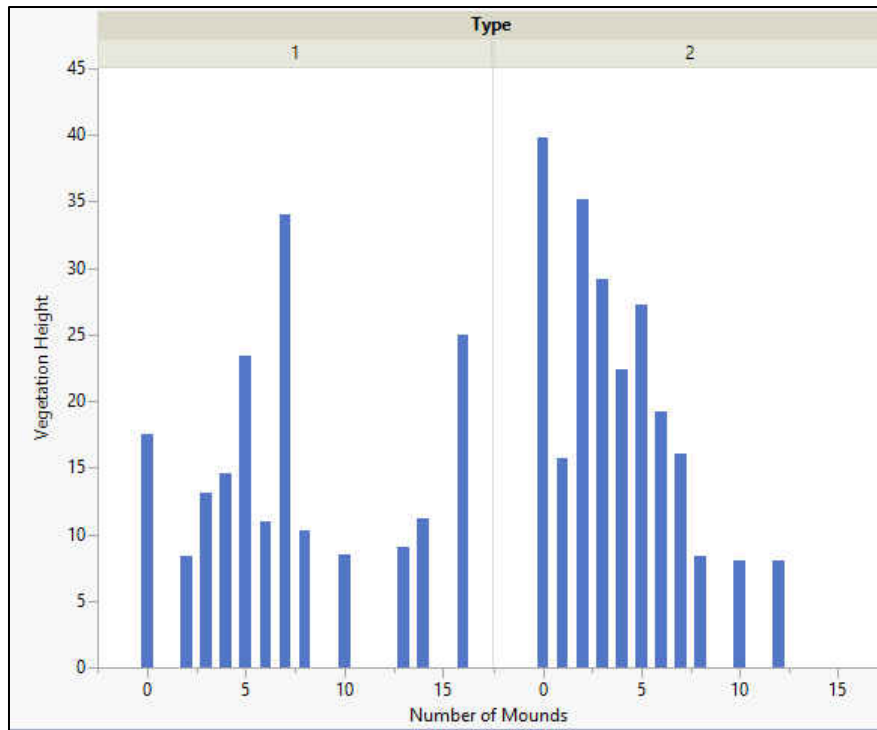


Figure 10: Vegetation height and number of mounds within each pasture type during the dry season (1 = intensive, 2 = semi-native).

Non-significance of Abiotic Variables and Distance to Edge Habitat

Despite evidence in the literature for the importance of soil temperature and soil moisture in *S. invicta* foraging and nest construction, neither were found to be significant predictors of density at the scale of our study. This is likely due to the relatively warm and moist conditions at nearly all sites, regardless of plot or pasture. This homogeneously high density across sites is supported by the work of LeBrun et al, which found *S. invicta* densities to be consistently high in disturbed and undisturbed mesic sites and dependent on the presence of disturbance in arid sites (Figure 7).

Although previous studies in Florida have found roadsides to be prime habitat for *S. invicta*, the less frequently traveled dirt roads within the pastures of the study site were likely not

significantly different enough from the surrounding pasture habitat to increase suitability. In addition, the extremely low elevation of the study site meant that not only were there over 600 wetlands within the boundaries of its 10,000 acres, but much of the pastures themselves flooded seasonally. This may create such a homogeneously moist environment that distances to water sources such as wetlands and ditches is of little significance.

The colonies surveyed in this study were not genetically analyzed to determine if their social form was monogyne (single queen) or polygyne (multiple queen). Monogyne ants have been found to colonize habitats in 2-3 times lower densities compared to polygyne (Macom 1996). Based on other studies within Florida, most habitats within the state are dominated by monogyne colonies (Porter 1993). Thus, the results of this study likely only apply to habitats with predominately monogyne populations.

Conclusions

Overall, within the wet pastures of south Florida, fluctuations of *S. invicta* density appears to be primarily driven by the recent history of soil-disturbing management activity within a pasture, but is otherwise largely consistent from pasture to pasture. Aeration and chopping of pastures disturbs the first 10-20 cm of soil, resulting in an exponential increase in *S. invicta* colonies at 1-2 years post-disturbance. If suppressing *S. invicta* is of interest to ranchers, alternative methods of controlling woody vegetation and increasing nutrients within soil should be used. The most viable alternative in this system would be prescribed burning, which functions similarly to chopping in its ability to reduce woody vegetation. Although the relationship between burning and *S. invicta* density is not entirely clear, a study by Hale et al. (2011)

demonstrated a reduction of *S. invicta* in burned areas. In systems with natural burn intervals, such as the wet and Dry Season prairie of this study, utilizing prescribed fire may both suppress *S. invicta* and improve overall ecosystem health. Continued research into the success of prescribed fire in maintaining low densities is needed in order to determine its efficacy in comparison to mechanical management methods

Beyond the impact of mechanical treatments, high grazing intensity measured in terms of dung pat density, likely provides a disturbance that promotes high *S. invicta* density. Thus, maintaining stocking densities within this study site that maintain dung pat densities below the mean (5.7 pats per 4m²) may aid in reducing *S. invicta* densities. Future research should investigate the roll that dung pats, and other temporary refugia (i.e. human structures on roadsides), play in protecting *S. invicta* from adverse conditions and maintaining their populations.

CHAPTER 3: THE EFFICACY OF THE BOILING WATER METHOD IN THE SUPPRESSION OF RED IMPORTED FIRE ANT (*SOLENOPSIS INVICTA*) POPULATIONS

Introduction

After the initial introduction of *Solenopsis invicta* to North America in the 1940's-1950's, extensive eradication efforts began in an attempt to prevent the continued spread of the species throughout the southeastern United States. Despite the broad application of chemically complex pesticides, the range of *S. invicta* continued to expand (Tschinkel 2006). Currently there are no feasible methods for eradication or control of fire ant populations across entire landscapes. For smaller areas such as backyards or golf courses, chemical treatment typically involves slow acting broadcast poison baits or rapidly acting contact insecticides, which may also impact non-target organisms (Plentovich et al. 2010). Meanwhile, non-chemical means of managing *S. invicta* populations have been poorly researched and few known options currently exist. This research focused on testing the efficacy of an alternative method for the treatment of individual fire ant mounds within pasture habitats; the boiling water technique.

The boiling water technique utilized in this study is a modification of a method first developed by Tschinkel and Howard (1980) in an effort to singularly eradicate *S. invicta* colonies and thus minimize impacts to the surrounding environment and non-target species. The process essentially involved boiling water, transporting it into the field and applying it directly to *S. invicta* mounds. Of the 14 colonies treated with this method, 57% experienced 80-100% colony death (Figure 11).

Treatment group	Colony Number	Mound Diameter	Approx. No of fire ant workers	Amount of rebuilding	Brood Surviving	Dead Piles	Kill estimate
Hot water (experimental)	1	63 cm	100's	+	-	+	3
	3	37	100's	+	+	+	3
	5	28	100+	+	-	+	3
	7	71	almost normal	+++	+++	++	1
	9	43	100's	+	-	+	3
	11	60	approx. 1000	++	-	++++	2
	13	31	100's	+	+	+	3
	15	37	approx. 100	-	-	+	4
	17	52	0	-	-	-	4
	19	40	approx. 1000	+	+	+	2
	21	34	normal	++++	+++	-	0
	25	31	approx. 1000	+	+	+	2
	27	38	less than 100	-	-	-	4
	29	44	more than 1000	++	++	++++	1
Cold water (controls)	14 colonies in all experimental group	similar to experimental group	normal	complete	normal	rare	0
							$\bar{x} = 2.5$

Figure 11: Figure from Tschinkel and Howard (1980) showing the mortality of *S. invicta* colonies treated with hot water. The kill estimates are as follows: 0 = no kill; 1 = some kill, but considerably less than 1/2; 2 = substantial fraction (1/2 to 3/4) of colony killed; 3 = most of colony killed, probably 80 to 95%; 4 = almost all to all killed, less than a hundred to zero survivors.

The second variation of the boiling water method utilized a kiln affixed to a pickup truck in order to expedite the boiling of water in the field (King and Tschinkel 2006, Tschinkel and King 2007). Water was then emptied into a bucket and carried to each mound in need of treatment. This improvement allowed for the control of larger populations of *S. invicta*, yet was still laborious and time intensive. Within the experimental plots used by King and Tschinkel 2006, two boiling water treatments were applied to a total of 155 mounds, resulting in no survival. After 10 months, the plots again were populated by 154 colonies; however these colonies were immature and smaller in size than the original mounds.

For this project, a third generation of the original design was created to allow for the rapid boiling of water in the field. A specialized trailer was constructed to ease the application of the water to the mounds through the use of a high capacity water tank, a pumping system, and a

30 m long hose. The improvements reduced the amount of time required to treat a mound to between 30 seconds and 2 minutes, depending on mound size and used approximately 5 gallons per minute. Although the amount of water applied to a single mound ranges from a mere 2.5 -10 gallons, it is possible that treating several mounds in a small area could result in an increase in surface soil moisture. It is unclear whether such a change in soil conditions may speed or delay future recruitment of *S. invicta* colonies into a plot.

To determine both the efficacy of the method and the impact of water addition alone on *S. invicta* survival and recruitment, we conducted an experiment within 24 plots on south Florida ranchland. The plot, totaling approximately 1 hectare in area, were randomly assigned one of 3 treatments: 1) control (no treatment) 2) sham control (room temperature water addition to mounds) 3) boiling water treatment to mounds. Seven months after treatment the plots were resurveyed and densities of *S. invicta* recorded. Based on the work of Tschinkel and King 2006 who saw a return to pre-treatment densities after 10 months, we expected to find densities of fire ants that were approximately 75% of the original density. We also hypothesized that the addition of water alone would increase soil moisture and due to the species affinity for soils with higher moisture levels, encourage recruitment into the plots (Lebrun et al. 2012).

Methods

Study Site

The study was conducted at MacArthur Agro-Ecology Research Center at Buck Island Ranch (hereafter MAERC) a 4,170-ha commercial cattle ranch, with approximately 3,000 cow-

calf pairs located in Lake Placid, Florida (Figure 12). MAERC is part of Archbold Biological Research Station.

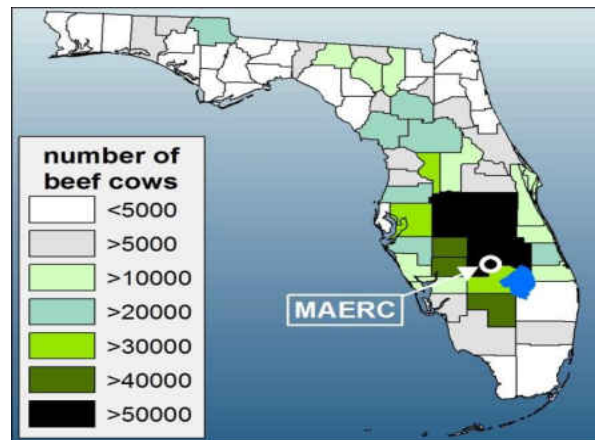


Figure 12: A map depicting the location of the study site at MAERC.

The subtropical climate has distinct wet and dry seasons and an average annual rainfall of ~130 cm. Most soils at MAERC are poorly drained, acidic, sandy spodosols, alfisols and entisols. MAERC is divided approximately 50:50 into two pasture types: intensively managed and semi-natural (Figure 13). Intensively managed pastures are planted with introduced Bahia grass (*Paspalum notatum*) and have been fertilized annually with NPK fertilizer for 20+ years prior to 1987. They have been fertilized only with N annually since 1987. In contrast, semi-natural pastures are not known to have ever been fertilized and are dominated by native grasses, such as bunchgrasses, (e.g., *Andropogon virginicus* and *Panicum* spp., *Axonopus* spp.). *Solenopsis invicta* colonies are commonly found throughout both pasture types.



Figure 13: Image showing the marked differences in the vegetation found in the semi-native pastures (on left) and intensively managed pastures found at MAERC.

Sampling

To conduct surveys of *S. invicta*, 6 pastures (3 each in semi-native and intensively managed pastures) were randomly selected from the 34 pastures available in the northern section of the MAERC. Because *S. invicta* density has been found to increase with increasing human disturbance, plots within pastures were placed using stratified random sampling by disturbance (i.e. proximity to road) (King and Tschinkel 2008). Approximately 1/3 of the 6 plots placed in each pasture were located in disturbed areas predicted to house a high density of fire ants; such as areas disturbed by road activity. The remainder of the sites were randomly located within the pasture.

Each of the 36 plots were 26×26 m in size and were surveyed entirely for fire ant mounds in May of 2014 (Figure 14). Surveys consisted of walking 13, 2 meter wide transects across each plot while scanning for mounds. Once the baseline ant abundance data had been collected, two

replicates of each of the following treatments were then assigned to each of the 6 plots within each pasture: kill (boiling water applied to each fire ant mound), sham control (room temperature water applied to each fire ant mound to test for impacts due to water addition alone) and control (no changes made to fire ant mounds). All treatments were applied in May of 2014 with a reapplication of the ‘kill’ treatment in July of 2014, which reduced densities to zero.

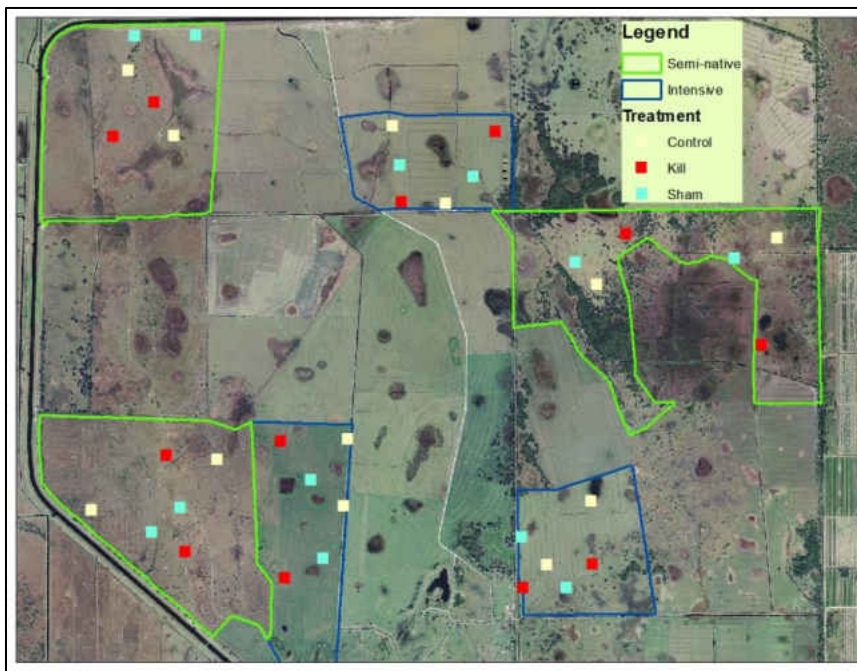


Figure 14: Map depicting the location of plots within semi-native (green outline) and intensively managed (blue outline) pastures. Treatments are denoted as red for plots where ants were killed, blue for sham plots (water at room temperature added), and white plots for control treatments.

Statistical Analysis

The study used a split-plot design with the variable ‘Pasture’ categorized as a random factor. Both the Shapiro-Wilk test (Shapiro 1965); R core team, 2014) and the Levene test (Levene, 1960) were utilized to evaluate parametric statistical assumptions. Based on these tests

as well as graphical exploration of the data, the assumptions of normality were met and a linear mixed effects model was selected as the best model for the data set.

The methods for model construction were based on Zuur et al. 2009. The fixed categorical variables used to construct the model were treatment (kill treatment, sham treatment or control) and pasture type (semi-native or intensively managed). The pasture type variable ('Type') was used as a fixed effect within the model in order to detect possible differences in the densities of *S. invicta* mounds due to the general habitat features of the two different pasture types. The model was calculated using Restricted Maximum Likelihood (REML).

Once the fixed and random structure of the model had been determined, the pre-treatment data were analyzed to determine if pre-treatment differences in *S. invicta* densities were present across the plots. Graphs were used to visually assess whether the assumptions of homogeneity of variance and normality of residuals were valid. The process of model construction, execution and validation was then repeated for the response variable "Mound Difference", which was calculated by subtracting the number of mounds present in plots after treatment from the number of mounds present before treatment.

Results

When comparing the average number of mounds within each plot before treatments, no significant differences could be found (Table 7). Thus, pre-treatment conditions were equitable across plots.

Table 7: Estimates for the number of *S. invicta* mounds within each treatment plot before treatments were applied. No significant differences between the 3 randomly assigned treatments were found.

	Estimate	Standard Error	DF	t-Value	Pr(> Z)
(Intercept/Control Treatment)	4.677589	1.72650	23	2.7092844	0.0125
Semi-native	-1.001758	2.049770	9	-0.4887173	0.6367
BWT Treatment	3.360652	1.698191	23	1.9789602	0.0599
Sham Treatment	2.716604	1.698191	23	1.5997051	0.1233

After the treatments were applied to the plots, the control and sham plots were not found to have significantly more or less colonies. In the plots where fire ants were killed, however, there were significantly fewer colonies 7 months after treatment (Table 8, Figure 15). This trend of significantly fewer ants in the plots where fire ants were killed was consistent across semi-native and intensive pastures.

Table 8: Estimates for the change in the number of fire ant mounds within each treatment plot after treatments were applied.

	Value	Std. Error	DF	t-Value	P
(Intercept)	1.477093	1.593500	27	0.926949	0.3622
Intensive Pasture	-1.201363	2.093630	5	-0.573818	0.5909
BWT Treatment	-4.288594	1.308374	27	-3.277806	0.0029
Sham Treatment	-0.205261	1.308374	27	-0.156883	0.8765

Overall the average number of ants per plot was 6.28 before treatment and 5.44 after treatment, or approximately 87% of the initial density. Although not significant, abundances in

the control and sham plots displayed an increasing trend after 7 months, while the treated plots saw a significant decline ($p = 0.0029$).

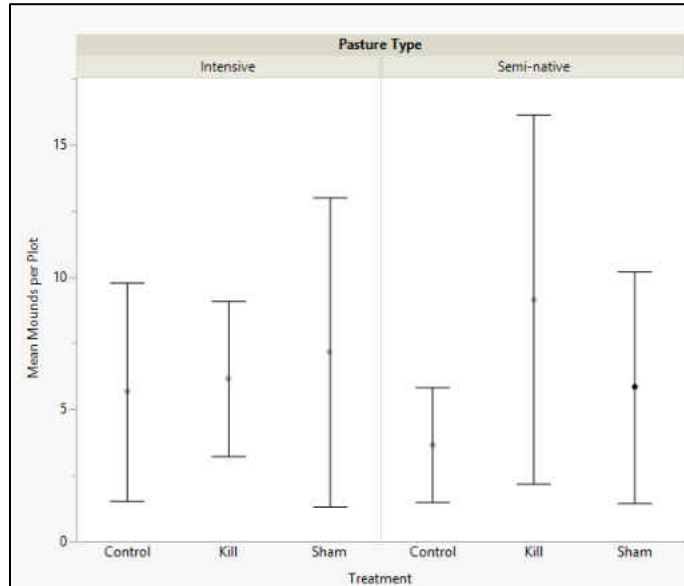


Figure 15: Mean total mounds per treatment within each pasture type before treatments were applied. Each error bar was constructed using a 95% confidence interval of the mean ($n = 6$).

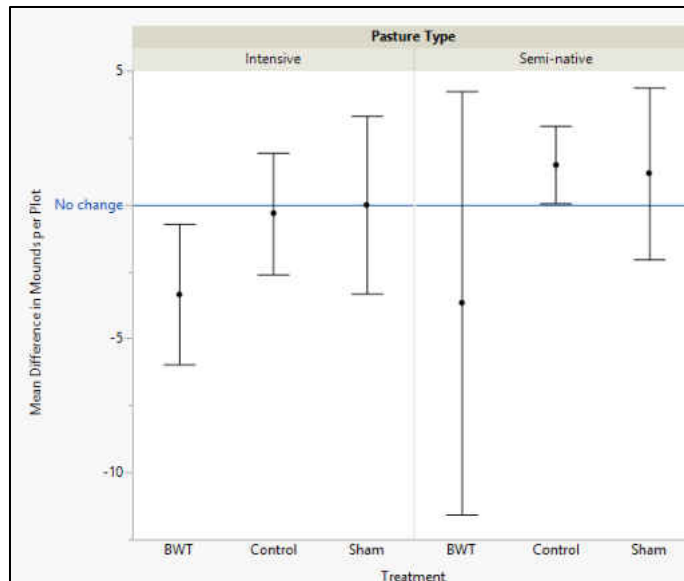


Figure 16: Mean difference in the number of mounds per treatment within each pasture type. Each error bar was constructed using a 95% confidence interval of the mean ($n = 6$).

Discussion

This study is one of only 2 studies that have been conducted on the efficacy of the boiling water method for removing *S. invicta* populations (Tschinkel and King 2007). In total, 7 months passed between the final treatment of mounds within the plots and the final survey. After that time, the sham and control plots had on average an increase of 0.5 mounds per plot, while the treated plots had an average decrease of 3.5 mounds per plot (Table 3). If the number of colonies within the treated plots continues to increase at the current rate, then the original densities would be achieved after approximately 13 months. Although the impact of pasture type was not significant within the model, the rate of return to pre-treatment densities would be faster for semi-native pastures (11.6 months) than intensive pastures (16.2 months). This trend may be related to the increased variability in habitat within semi-native pastures compared to intensive pastures, which are primarily dominated by exotic Bahia grass, *Paspalum notatum*.

The application of the room temperature water within the sham plots was designed to determine whether ant populations decrease due to the disturbance of flooding alone or increase due to an increase in soil moisture. Neither seems to be the case, as the sham plots were no different from the control plots in terms of mound abundance. Concerns we had about indirectly improving conditions for *S. invicta* to recolonize plots by increasing soil moisture through the water additions were also quelled. Sham water additions showed no positive correlation with ant abundances throughout the study.

Conclusion

Although utilizing the hot water treatment method requires acquiring specific equipment (portable water heater, pressure sprayer etc.), doing so makes applying the treatment to mounds only somewhat more labor intensive than traditional pesticide treatments to individual mounds. Each mound treated within this study required 30 seconds - 2 minutes of water application, depending on mound size, before eradication of the colony was achieved. This is significantly faster than previous work done with the boiling water method that utilized a kiln and bucket for application of the water. Furthermore, killing ants with poisons may unintentionally impact non-target invertebrates or predators (Markin et al. 1974, Plentovich et al. 2010). This is of special concern for experimental work where impacts on other non-target insect populations would negatively impact the outcome of the experiment. Thus, this method is an ideal, ecologically responsible, technique for controlling *S. invicta* populations.

CHAPTER 4: EXPERIMENTAL INVESTIGATION OF DUNG BEETLE AND FIRE ANT INTERACTIONS

Introduction

Grazing animals, particularly cattle, in Florida deposit over 20 million metric tons of dung per year, making dung a significant non-point source of pollution (extrapolated from Fincher, 1981). Dung that is not degraded may be leached into water bodies where it both releases pharmaceuticals excreted by livestock and contributes to the eutrophication of waterways (Sanderson et al. 2007, Carpenter et al. 1998). Dung that remains un-degraded on the surface of a pasture also provides an incubation site for the pests and parasites that infect both humans and livestock (Fincher, 1981). In ecosystems on every landmass except Antarctica, degradation of this dung occurs naturally, largely due to a diverse group of beetles (Order Coleoptera) in the families Scarabaeidae and Geotrupidae (hereafter dung beetles). The consumption and relocation of dung by dung beetles provides a suite of ecosystem services that lead to a reduction in the leaching of dung into waterways, a reduction in livestock pests and parasites and an increase in the recycling of dung nutrients into the soil (Losey and Vaughan, 2006). Therefore, the optimization of these ecosystem services provided by dung beetles is a priority for not only the protection of terrestrial and aquatic biota, but the cattle industry itself.

Of the services provided by dung beetles, the suppression of parasites that impact livestock has become of increasing importance as chemical means of parasite control have lost their efficacy due to the development of resistance (Gill et al. 1995). Roundworms of the family Strongyloididae are of particular economic importance as millions of dollars are spent each year on specifically managing infections (Corwin, 1997). As adults, roundworms and other

gastrointestinal parasites live within the gut of their host animal and release eggs into the animals' feces (Figure 17). Once the eggs hatch into larvae, they spend 5-30 days (depending on weather conditions) incubating within the dung pat. After their 3rd molt they are known as "L3" larvae and are infective. L3 larvae then migrate up to 30cm horizontally through the vegetation surrounding the dung pat where they await consumption in order to complete their lifecycle within the gut of the animal (Fincher 1973).

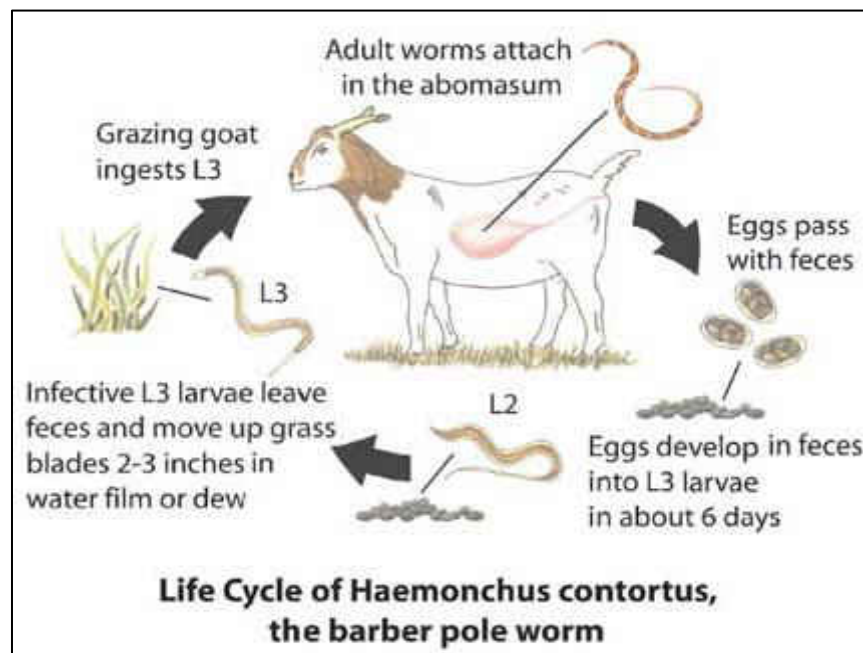


Figure 17: A depiction of the life cycle of *Haemonchus contortus*, a common gastrointestinal parasite of livestock in Florida (figure from the University of Arkansas).

Several studies have shown that dung beetle activity in pastures negatively impacts parasite abundance (Bryan, 1976, Fincher, 1973). For example, within a temperate pasture, Bryan (1976) found that control dung pats had 50 times more livestock parasitic nematode larvae than pats with 10-30 beetle pairs. The exact mechanism by which dung beetles interrupt the parasite lifecycle is not entirely clear. Larvae are likely killed in one of the following ways: 1) dung beetle consumption and maceration of larvae (Miller, 1961), 2) aeration and dehydration of

the dung pat through the tunneling activity of (Mfitlodze and Hutchinson, 1988), 3) physical removal of larvae through the burial of dung by dung beetles (Edwards and Aschenborn, 1987).

Furthermore, the mechanism utilized for killing parasites may be dependent upon which of three reproductive strategies is employed by the beetle species. Rolling and tunneling dung beetles for example, remove dung from the dung pat, and shape it into balls with a single egg placed within the center. These reproductive dung balls are then buried in belowground tunnels excavated by the adult beetle (Simmons and Ridsdill-Smith, 2011). Some researchers have theorized that the amount of dung buried by tunnelers and rollers is directly proportional to the number of parasites killed within the dung pat. However, a study by Coldham (2011) allowed dung beetles to bury dung and compared the survival of parasites to dung buried by human-hand. The results were surprising as parasites emerged from the human buried dung and not from the dung buried by beetles. This suggests that the impact of burial may not be important for parasite survival and more research is needed to determine what other mechanisms may exist.

Dwelling dung beetles, unlike rolling and tunneling dung beetles, lay eggs within the dung pat and do not bury dung. Contrary to the results of Coldham (2011) study which primarily utilized tunneler species, a recent study by Chirico et al. 2003 found that in the dweller-dominated pastures of Europe, dung beetle activity, under moist environmental conditions, actually increased parasite survival (Figure 18). This was hypothesized to be related to aeration of the dung pat by dung beetle movement. Parasite eggs require exposure to oxygen and moisture in order to hatch from egg to larvae. Dung beetle activity during moist environmental conditions allowed air to penetrate beyond the crust of the dung pat and increased parasite hatching. During arid conditions, this relationship was not observed. Whether this relationship is maintained when

tunneler and roller species are present is doubtful, however further studies are needed to clarify the relationship between dung beetles and parasite abundances.

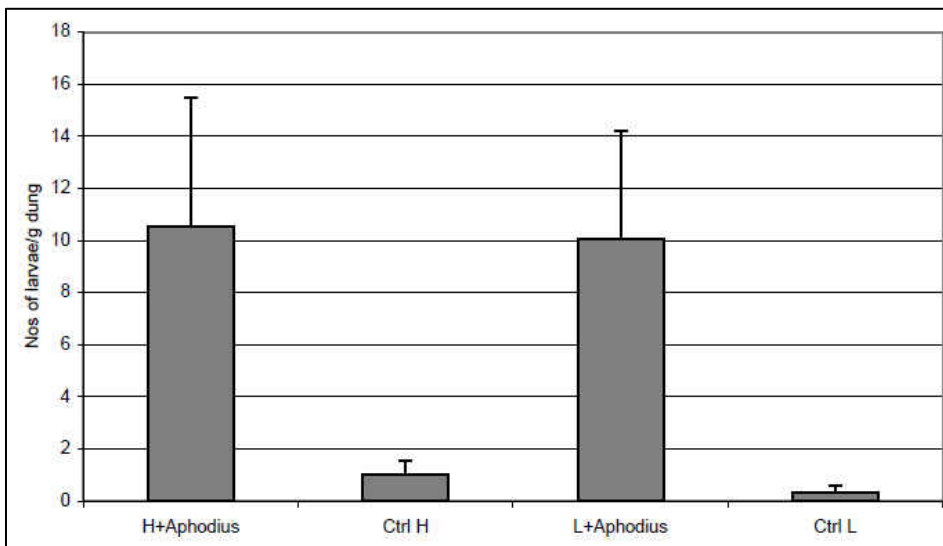


Figure 18 : Figure from Chirico et al. 2003 showing the mean number of gastrointestinal larvae per gram (lpg) recovered after 12 days from cattle dung that contained 250–600 eggs per gram (H) and 100 eggs per gram (L), respectively, with presence of *Aphodius rufipes* and *Aphodius scybalarius* and where no dung beetles were subjected to the dung (Ctrl).

Although dung beetles are typically a dominant species within dung pats, the introduction of non-native species may alter their dominance in the dung pat microcosm. In Florida, the introduction of the red imported fire ant, *Solenopsis invicta*, is of interest as the species has been found to heavily utilize dung pats for both nest construction and foraging (Summerlin et al., 1984). Lab experiments by Summerlin et al. 1984 found that the foraging of *S. invicta* within dung pats reduced the survival of the larvae of the hornfly *Haematobia irritans*, a hematophagic pest of livestock. Thus, the potential interaction between *S. invicta* and other dung dwelling insects is unclear. *S. invicta* workers may be depredating, competing with, or simply not impacting dung beetles within dung pats. Due to the importance of the ecosystem services

provided by dung beetles, research into the dynamics of the interactions between *S. invicta* and dung beetle communities, and their subsequent impact on ecosystem services, is greatly needed.

This study focuses on understanding this interaction by evaluating how the presence or absence of the non-native *Solenopsis invicta* and the presence or absence of dung beetles influence 1) the rate of dung degradation and 2) parasite suppression within pastures. I hypothesized that dung beetle access to dung pats will both decrease the size of dung pats and the parasite survival, as has been found in studies conducted within tropical systems. I also hypothesized that *Solenopsis invicta* will not influence the size of dung pats, but foraging activity within dung pats will increase pat desiccation, resulting in fewer parasites.

Table 9: Research questions, hypotheses and predictions for the amount of dung degraded after exposure to *S. invicta* alone, dung beetles alone and both groups combined.

Question	Hypothesis	Prediction
Do differences in pasture type impact the rate of dung degradation?	If insect-free dung primarily degrades due to weathering (Anderson et al. 1984), then no difference will be found since rainfall and UV exposure will be similar across pasture types.	No change in dung degradation
Does <i>S. invicta</i> presence increase or decrease the rate of dung degradation?	Although <i>S. invicta</i> forage for invertebrates and seeds in dung, the amount removed will not be a significant portion of organic matter (Summerlin 1973).	No change in dung degradation
Does dung beetle presence increase or decrease dung degradation rate?	Dung beetles increase the rate of dung degradation in tropical climates due to the burial of reproductive dung balls (Slade 2007).	Increase in dung degradation
Is the interaction between <i>S. invicta</i> and dung beetle communities influencing dung degradation rate?	If <i>S. invicta</i> are preying upon or competitively suppressing dung beetles, dung degradation will decrease in plots with fire ants and dung beetles compared to plots with only dung beetles.	Decrease in dung degradation when both are present.

Table 10: Research questions, hypotheses and predictions for the number of surviving gastrointestinal parasite larvae after exposure to *S. invicta* alone, dung beetles alone and both groups combined.

Question	Hypothesis	Prediction
Do differences in pasture type impact the survival of parasites?	If insect-free dung primarily degrades due to weathering (Anderson et al. 1984), then no difference will be found since rainfall and UV exposure will be similar across pasture types.	No change in parasite survival
Does <i>S. invicta</i> presence increase or decrease the survival of parasites?	Desiccation of dung pats reduces parasite emergence (Rossanigo and Gruner 1995). Foraging tunnel activity of <i>S. invicta</i> is within dung pats, may lead to an increase in the permeability and desiccation of the pat.	Decrease in parasite survival
Does dung beetle presence increase or decrease the survival of parasites?	Dung beetles have been found to decrease the survival of parasites (Fincher 1981), however the mechanism for this ecosystem service is not clear.	Decrease in parasite survival
Is the interaction between <i>S. invicta</i> and dung beetle communities influencing the survival of parasites?	If <i>S. invicta</i> are preying upon or competitively suppressing dung beetles, parasite survival will increase in plots with fire ants and dung beetles compared to plots with only dung beetles.	Increase in parasite survival when both are present.

Methods

Study Site

The study was conducted at MacArthur Agro-Ecology Research Center at Buck Island Ranch (hereafter MAERC) a 4,170-ha commercial cattle ranch, with approximately 3,000 cow-calf pairs located in Lake Placid, Florida (Figure 3). MAERC is part of Archbold Biological Research Station.

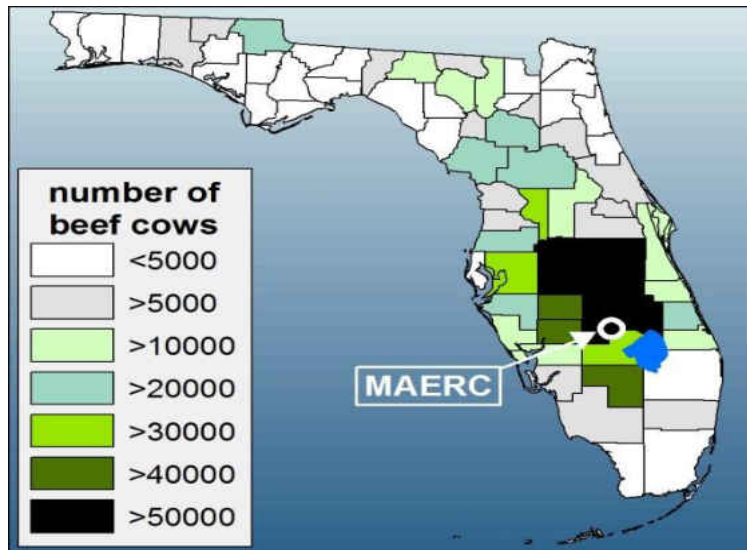


Figure 19: A map depicting the location of the study site at MAERC.

The subtropical climate has distinct wet and dry seasons and an average annual rainfall of ~130 cm. Most soils at MAERC are poorly drained, acidic, sandy spodosols, alfisols and entisols. MAERC is divided approximately 50:50 into two pasture types: intensively managed and semi-natural (Figure 20). Intensively managed pastures are planted with introduced Bahia grass (*Paspalum notatum*) and have been fertilized annually with NPK fertilizer for 20+ years prior to 1987. They have been fertilized only with N annually since 1987. In contrast, semi-natural pastures are not known to have ever been fertilized and are dominated by native grasses, such as bunchgrasses, (e.g., *Andropogon virginicus* and *Panicum* spp., *Axonopus* spp.). *Solenopsis invicta* colonies are commonly found throughout both pasture types.



Figure 20: Image showing the marked differences in the vegetation found in the semi-native pastures (on left) and intensively managed pastures found at MAERC.

Sampling

In order to establish the experimental plots, 6 pastures (3 each in semi-native and intensively managed pastures) were randomly selected from the 34 pastures available in the northern section of the MAERC. Because *S. invicta* density has been found to increase with increasing human disturbance, plots within pastures were placed using stratified random sampling by disturbance (i.e. proximity to road) (King and Tschinkel, 2008). Approximately 1/3 of the 6 plots placed in each pasture were located in disturbed areas predicted to house a high density of fire ants; such as areas disturbed by road activity. The remainder of the sites were randomly located within the pasture (Figure 21).

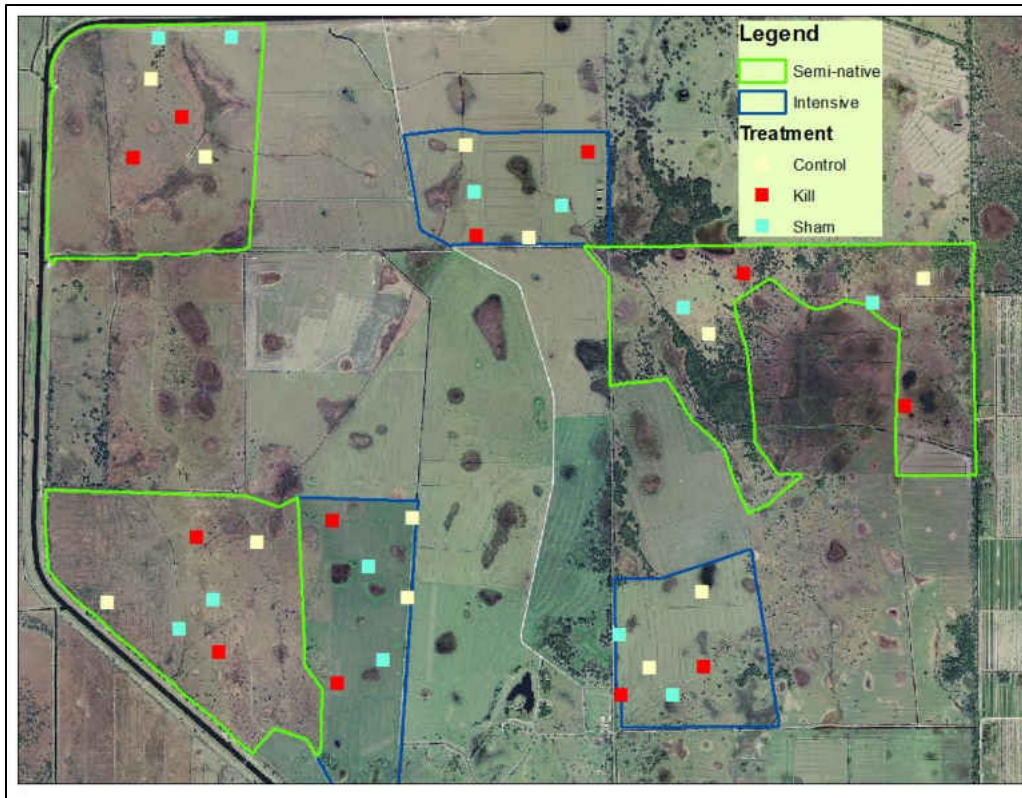


Figure 21: Map depicting the location of plots within semi-native (green outline) and intensively managed (blue outline) pastures. Treatments are denoted as red for plots where ants were killed, blue for sham plots (water at room temperature added), and white plots for control treatments.

Each of the 36 plots were 26×26 m in size and were surveyed entirely for fire ant mounds in May of 2014. Surveys consisted of walking 13, 2 m wide transects across each plot while scanning for mounds. Once the baseline ant abundance data had been collected, two replicates of each of the following treatments were then assigned to each of the 6 plots within each pasture: kill (boiling water applied to each fire ant mound), sham control (room temperature water applied to each fire ant mound to test for impacts due to water addition alone) and control (no changes made to fire ant mounds). All treatments were applied in May of 2014 with a reapplication of the ‘kill’ treatment in July of 2014.

Dung beetle abundance and community composition were also tracked bi-monthly from January 2014 until December 2014 through dung-baited pitfall traps at the 36 experimental sites. Each site contained two dung-baited pitfall traps that were left open for a 24 hour period once each month. Because trapping required killing many of the beetles collected, trapping of beetles was suspended from October to November 2014 when the experiment was actively running to reduce the likelihood of locally 'trapping out' the dung beetle community. This data set established the presence of a variety of species in all pastures included in the experiment.

Once the baseline data on the abundance of *S. invicta* and community composition of dung beetles had been established, experimental exclosures were constructed at the center of every plot (n=36). These exclosures were 5m x 3m in size and were surrounded by 4 foot high hog fencing in order to prevent disruption of the experiment by cattle. Dung was then collected the day before opening the experiment from a single herd of cattle that, unlike other cattle at the study site, had not been treated with dewormer (LongRange™, eprinomectin) for the previous 40 days. This was done to prevent negative impacts from the toxicity of dewormers excreted in cattle dung. Once the dung was collected it was homogenized and 2 standard dung pats were formed in the lab and allowed to air dry. Pats were formed within a Styrofoam mold, 15 cm in diameter and 4 cm tall. These dimensions are approximately the dimensions of the average dung pat from U.S. beef cattle and have been used in other studies (Slade et al. 2007). Once in the field, a grid of 2 X 4 dung pats was placed inside of the exclosure and a minimum distance of 30cm maintained between pats in order to prevent overlap of parasite migration (Marley 2006, Figure 22). In total, this placed 8 pats inside of each exclosure, only 4 of which were used for the parasite data while all 8 were later used to assess dung degradation. Each of the pats were then

randomly assigned to a dung beetle treatment. Those pats that were assigned a ‘No dung beetle’ treatment had a 15cm X 15cm X 10cm metal cage over the top of them. The 2 cm wide mesh ensured that the head of a *S. invicta* worker could still fit through the mesh, but the average dung beetle could not.

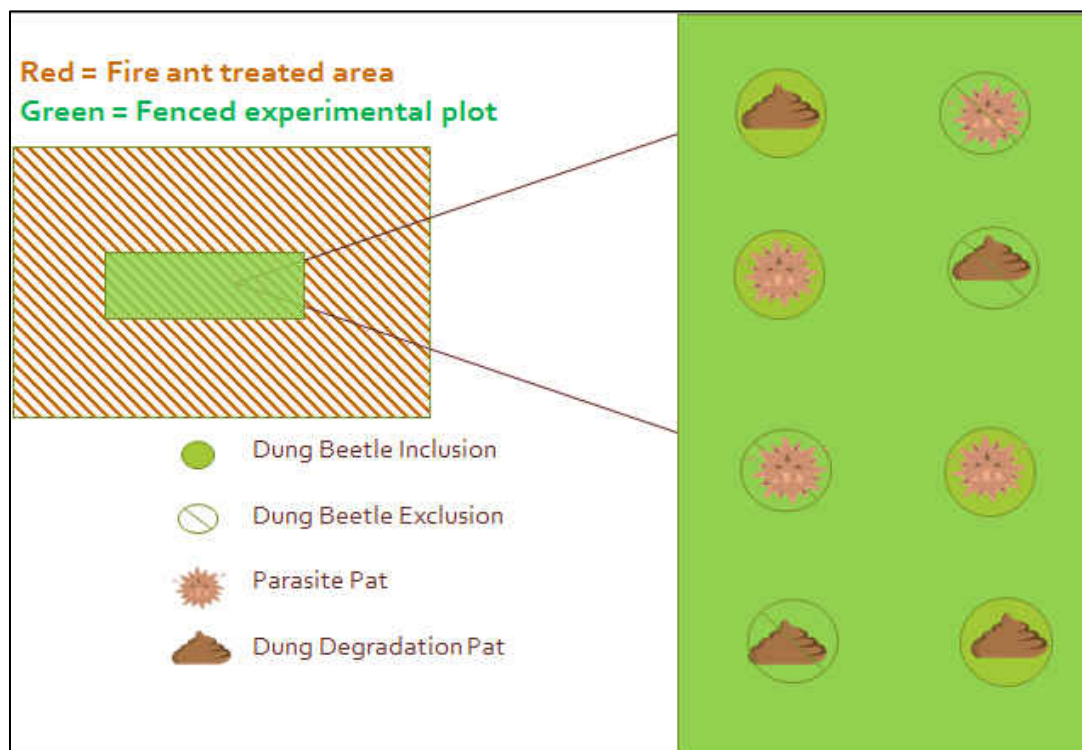


Figure 22: Diagram depicting the experimental design of a single plot within a pasture. The entire plot was randomly assigned one of 3 *S. invicta* treatments: kill, sham or control. The center of each plot was fenced to prevent disruption from cattle and 8 dung pats were placed in 2 rows. Pats were then randomly assigned one of 2 dung beetle treatments: fenced (dung beetles excluded) or unfenced (dung beetles included).

Once placed inside of the plot, the dung pats were left in the field for 30 days. After that time the pats were collected and returned to the lab where they were dried at 70°C for 48 hours and weighed to assess the loss of organic material. During the dung pat collection, a 10cm ring of grass adjacent to each dung pat was clipped, bagged into plastic bags and returned to the lab for parasite analysis.

Once in the lab, a procedure was initiated to begin the extraction of the infective gastrointestinal L3 parasite larvae from the grass samples. This procedure consisted of 3 major steps: 1) Extraction of parasites from grass samples, 2) Centrifugation of sample to 1 ml or less, and 3) Identification of the parasite under a microscope (Boom and Sheath, 2008, Demeler et al., 2012). To extract parasites the grass samples were soaked in a 0.5% tween detergent solution for 48 hours. Samples were then double sieved with a 100 uL sieve to remove vegetation. The remaining liquid was placed into 50ml centrifuge tubes and centrifuged at 2,740 rpm for 5 min. The supernatant was removed and the process was repeated until the sample was reduced to 1 ml. The samples were stored at 4°C until they could be scanned. Before scanning, the sample was homogenized and 10 uL placed onto the center of a slide. Two drops of iodine and a coverslip added. After sitting for 2 minutes, the entire area under the coverslip was scanned for parasites. Any parasites found were photographed using a microscope camera (Figure 23). While it is very difficult to identify L3 larvae to species, it is much simpler to simply differentiate between parasitic larvae and the free ranging larvae within vegetation that feed on plant matter, fungi and bacteria. Thus, all parasites photographed were later identified as ‘parasitic’ or ‘free ranging’ for data analysis.



Figure 23: Examples from 2 samples collected from the grass samples around dung pats. On the left is a free-living nematode characterized by a bulbous esophagus (green arrow) and lack of external sheath. On the right is an infective larvae characterized by ribbing along the body from the presence of a sheath as well as a lack of the bulbous esophagus.

Statistical Analysis

Separate models were constructed to first analyze two different response variables: 1) the number of parasites that survived (denoted as larvae per gram, LPG) from each dung beetle treatment within each plot and 2) the average size of a dung pat (in grams) remaining for each dung beetle treatment within each plot. The data collected for this study were nested at three different levels. The primary units were the pastures which were randomly selected from the larger pasture-type blocks. Plots were then sampled within each pasture that were subjected to three treatments (sham, control, and kill). Lastly, the dung pats within each plot were treated with either dung beetle presence or absence.

Both the Shapiro-Wilk test (Shapiro, 1965) (R core team, 2014) and the Levene test (Levene, 1960) were utilized to evaluate statistical assumptions for both parasite and dung degradation analysis. Due to the results of these tests, the hierarchical design of the study and the count response variable, a generalized mixed linear model with a negative binomial distribution was utilized for the parasite data. The variables ‘Pasture’ and ‘Plot’ were assigned as random factors due to the split-split plot design of the experiment. The following fixed factors were also included: pasture type (semi-native or intensive), plot treatment (fire ants killed, sham, or control) and dung pat treatment (fenced or unfenced) (Equation 1). Fixed terms were then sequentially removed from the model and AICc values compared to select the most parsimonious model. All analyses were done in R, using packages “glmmadmb” and “bblme” (R Core Team 2014).

$$\begin{aligned} \text{Number of Parasites} &\sim \text{Pasture Type} * \text{Fire Ant (Plot)Treatment} * \\ \text{Dung Beetle (Pat)Treatment} &+ \text{random terms (Pasture|Plot)} \end{aligned} \quad (1)$$

For the dung data, the Levene and Shapiro tests revealed normal distributions of the data set even within the subsets of the categorical explanatory variables. Thus, a linear mixed model was used with ‘Pasture’ and ‘Plot’ assigned as random factors due to the split-split plot design of the experiment. As with the parasite data, the following fixed factors were utilized in model construction: pasture type (semi-native or intensive), plot treatment (fire ants killed, sham, or control) and finally dung pat treatment (fenced or unfenced) (Equation 2). Fixed terms were then sequentially removed from the model and AICc values compared to select the most parsimonious model. All analyses were done in R, using packages “lme4” and “bblme” (R Core Team 2014).

$$\begin{aligned} \text{Size of Dung Pat} &\sim \text{Pasture Type} * \text{Fire Ant (Plot)Treatment} * \\ \text{Dung Beetle (Pat)Treatment} &+ \text{random terms (Pasture|Plot)} \end{aligned} \quad (2)$$

Results

Dung Beetle Abundances

Overall, dung beetles were found to be present within the pastures year-round, with tunneling species making up the majority of beetles collected in all months (Table 11). This indicates that although beetles were not trapped during the experiment in order to prevent potential interference, beetles were likely still active. Due to pasture size, there were 28 pitfall trapping sites in the intensive pastures and 35 in the larger semi-native pastures. Thus, instead of reporting the raw dung beetle abundances in Table 12, the number of dung beetles found within each pasture type was divided by the number of trapping sites, in order to correct for the difference in trapping site number.

Table 11. Abundance of dung beetles collected during dung-baited pitfall trapping and divided by reproductive functional group.

Month	Dweller	Tunneler	Roller
January	60	336	0
March	34	308	3
May	33	195	1
June	8	227	5
July	47	555	4
September	55	255	11
December	94	790	0

Table 12. The average number of dung beetles collected in each reproductive functional group per trapping site in intensive and semi-native pastures from January to December 2014.

Intensive Pasture				Semi-native Pasture			
Month	Dweller	Tunneler	Roller	Month	Dweller	Tunneler	Roller
January	2.13	3.79	0.00	January	0.26	7.00	0.00
March	0.67	6.00	0.04	March	0.51	4.69	0.06
May	0.54	2.21	0.04	May	0.57	4.06	0.00
June	0.11	1.75	0.14	June	0.14	5.09	0.03
July	0.89	3.75	0.00	July	0.63	12.86	0.11
September	1.36	3.07	0.00	September	0.49	4.83	0.31
December	2.64	20.82	0.00	December	0.57	5.91	0.00

Parasite Suppression

The dung beetle treatment (fenced to exclude dung beetles or unfenced) was the only term found to have a significant impact on the number of gastrointestinal parasites surviving incubation within dung pats ($p = 0.025$) (Table 13). Plots with dung beetles present, regardless of pasture type or fire ant treatment, had fewer parasites (Figure 24). The semi-native pastures also showed a trend towards decreased parasites, however this was not significant ($p = 0.148$).

Table 13: Estimates for the full model including the fixed factors Pat Treatment (fenced or unfenced), Plot Treatment (Kill, Sham or Control) and Pasture Type (Semi-native or intensive) on the survival of infective parasite larvae. Total number of observations = 59. Random effect variance for the random effect 'Pasture' = 0.117; std. dev. = 0.342. Nested random effect 'Pasture:Plot' variance = 1.128e-07; std. dev. 0.0003. Negative binomial dispersion parameter = 0.46171; std. error = 0.11073. Log likelihood = -208.37.

	Estimate	Std. Error	Z-value	Pr(> z)
(Intercept) DB Absent	3.5283	0.6496	5.43	5.6E-08
DB Present	-0.7507	0.8669	-0.87	0.39
No FA	-0.1744	0.8613	-0.2	0.84
FA Sham	0.0469	0.8745	0.05	0.96
Semi-native Pasture	-0.4963	1.0247	-0.48	0.63
DB Present:FA Absent	-0.2132	1.2562	-0.17	0.87
DBPresent:FA Sham	-0.6002	1.2204	-0.49	0.62
DB Present:Semi-native Pasture	-13.8295	156.97	-0.09	0.93
FA Absent:Semi-native Pasture	-0.0653	1.3615	-0.05	0.96
FA Sham:Semi-native Pasture	-0.2372	1.3628	-0.17	0.86
DB present:FA AbsentL:Semi-native Pasture	14.905	156.97	0.09	0.92
DB Present:FA Sham:Semi-native Pasture	13.8333	156.97	0.09	0.93

Table 14: The model determined by AICc rank to most parsimoniously predict the survival of parasite larvae: Count~ Pat Treatment + Pasture Type + (1|Pasture/Plot). Total number of observations = 59. Random effect variance for the random effect 'Pasture' = 1.786e-075; std. dev. = 0.004. Nested random effect 'Pasture:Plot' variance = 1.125e-07; std. dev. 0.0003. Negative binomial dispersion parameter = 0.36575; std. error = 0.077812. Log likelihood = -213.887.

	Estimate	Std. Error	Z-value	Pr(> z)
(Intercept)	3.563	0.356	10	<2e-16
DB Present	-0.983	0.438	-2.25	0.025
Semi-native Pasture	-0.645	0.446	-1.45	0.148

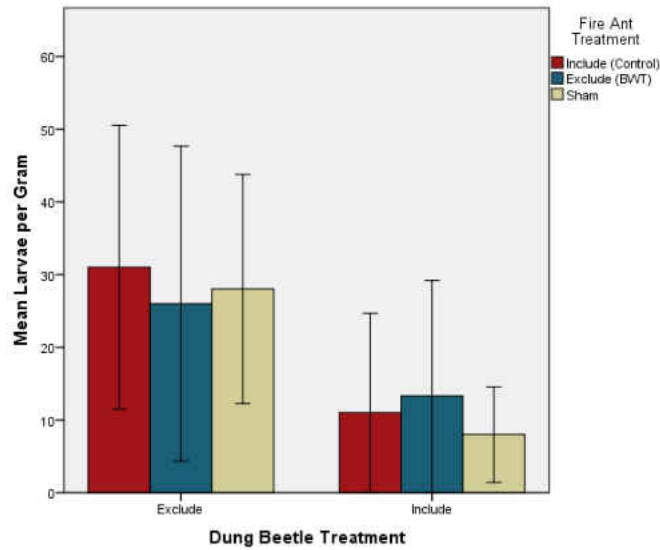


Figure 24: Mean parasites per pat treatment (fenced pats excluded dung beetles and unfenced pats included dung beetle activity). Fewer larvae per gram (LPG) were recovered from dung pats exposed to dung beetle activity. Error bars are 95% confidence intervals.

Dung Degradation

The results from the dung data displayed no significant relationships (Table 15). There was a general trend of decreased dung pat size in the unfenced control plots (Figure 25). In comparison, the unfenced kill and sham plots showed a trend towards larger pat sizes. Overall however, no terms were found to significantly predict dung pat size.

Table 15: Estimates for the full model including the fixed factors Pat Treatment (fenced or unfenced), Plot Treatment (Kill, Sham or Control) and Pasture Type (Semi-native or intensive) on the survival of infective parasite larvae.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	65.8125	5.23696	50	12.5669	0
fPlotK	2.7875	6.41394	2	0.4346	0.7062
fPlotS	1.1125	6.76088	2	0.16455	0.8844
fPatFenced	6.3625	6.76088	50	0.94108	0.3512
fPatUnfenced	-0.0778	6.04712	50	-0.0129	0.9898
fType2	1.57917	6.76088	50	0.23357	0.8163
fPlotK:fPatFenced	-14.488	8.81511	50	-1.6435	0.1066
fPlotS:fPatFenced	0.38333	9.56133	50	0.04009	0.9682
fPlotK:fPatUnfenced	-0.7115	7.62341	50	-0.0933	0.926
fPlotS:fPatUnfenced	4.29278	8.11306	50	0.52912	0.5991
fPlotK:fType2	-4.4208	9.31924	50	-0.4744	0.6373
fPlotS:fType2	4.02708	8.81511	50	0.45684	0.6498
fPatFenced:fType2	-2.7563	8.81511	50	-0.3127	0.7558
fPatUnfenced:fType2	-0.7764	7.99959	50	-0.0971	0.9231
fPlotK:fPatFenced:fType2	8.73542	12.8279	50	0.68097	0.499
fPlotS:fPatFenced:fType2	-9.3764	12.4665	50	-0.7521	0.4555
fPlotK:fPatUnfenced:fType2	4.76234	11.1328	50	0.42778	0.6706
fPlotS:fPatUnfenced:fType2	-4.1377	10.7143	50	-0.3862	0.701

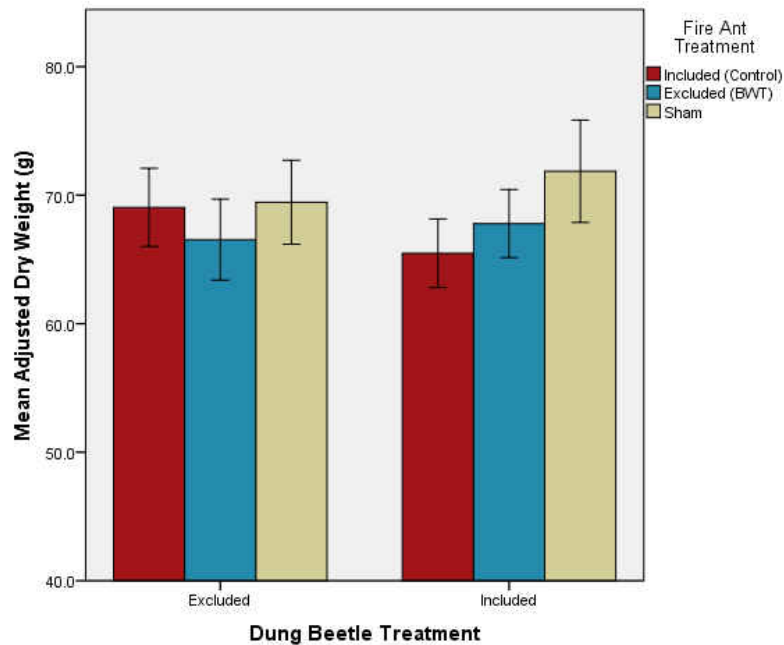


Figure 25: Depiction of the impact of the interaction between pasture type, fire ant treatment and dung beetle treatment on the weight of dung. No significant effect was found. Error bars are 95% confidence intervals of the mean.

Discussion

Impacts of Dung Beetles

Overall, parasite survival was significantly reduced in the presence of dung beetles, regardless of pasture conditions and fire ant treatments. While dung beetles have been found to suppress gastrointestinal parasites of livestock in previous studies in temperate systems, this is the first study in a sup-tropical climate to document the same effect. The mechanism by which dung beetle activity results in this reduction is not entirely clear. However, the hypothesis that dung beetle activity reduces parasite survival indirectly through the burial of dung was not supported because the amount of dung in grams lost to dung beetle activity was not significant. This is supported by a recent study by Coldham (2011) where parasite survival was compared in

samples of dung buried by human hands vs. dung beetle buried dung. Surprisingly, the human-buried dung had rates of parasite survival similar to un-buried dung, while the beetle buried dung had significantly less survival. Together these studies suggest burial is not the primary mechanism driving parasite suppression.

If dung beetles are not primarily reducing parasite survival through dung burial, what other mechanisms may be responsible? While adult dung beetles bury dung, they also consume it through mouthparts that filter the liquid fraction from the organic matter (Miller, 1961). While this may directly kill parasite larvae through physical damage, feeding activity may also lead to desiccation of the dung pat which reduces the ability of the moisture-loving parasites to complete their molts and reach the infective stage. Additional desiccation may also result from dung beetle movement through the pat. While aeration is advantageous to parasites during hypoxic conditions in the center of the pat, aeration may also lead to desiccation of the pat when conditions external to the dung pat are arid or hot (Chirico et al. 2003).

Impacts of Solenopsis invicta

No evidence was found to indicate that *S. invicta* presence impacts the activity of dung beetles or the survival of parasites in the absence of beetles. Because natural densities of ants were used in this experiment, it is possible that *S. invicta* densities were simply too low to detect an effect with this experimental design. Overall, *S. invicta* densities were found to be on average 70 mounds per hectare, which is relatively low in comparison to the highest densities recorded in Texas of 600 mounds per hectare (Steele unpublished, Vogt et al. 2003).

It is also possible however, that the time at which dung beetles and *S. invicta* foragers colonize dung pats is vastly different, leading to a temporal partitioning of the pat. Dung beetles have been found to enter dung pats from almost immediately after dung is deposited up to 48 hours later (Gittings and Giller, 1998). However, the vast majority of visits are within the first 4 hours after the pat is deposited on the pasture surface. *S. invicta*, however may not be able to tolerate the moist conditions of the pat as easily. A study by Xu et al. 2009, showed that *S. invicta* workers exposed to a sudden increase in soil moisture inside of the nest suffered high mortality as a result. Whether this relationship holds true for foraging sites is unclear, but it may lead to *S. invicta* workers only accessing the dung pats after enough time has passed for moisture to sufficiently decrease. There is no indication that *S. invicta* is averse to preying upon dung beetles, as beetles were frequently dismembered by the ants in pitfall traps unrelated to this experiment (Steele unpublished).

Potential Impacts of Weather Events

Due to the low elevation of the pastures included in this study as well as the wet summer climate of south Florida, many of the pastures experienced seasonal flooding each year during the summer months. This flooding could last several days to several weeks. Because dung beetles form brood chambers beneath the surface of the soil, it is likely that the larvae experienced inundation during these flood events. Many burrowing insects have to been found to possess adaptations that allow them to survive periods of hypoxia or anoxia due to flooding (Hoback and Stanley 2001). For example the larvae of the tiger beetle, *Cicindela togata* found throughout North America, has been shown to withstand inundation of its below ground brood

chambers for up to 6 days (at 25°C) by depressing its metabolism and switching to an anaerobic pathway for energy metabolism (Hoback et al. 1998). Unfortunately, no such research has been conducted on the larvae of dung beetle species and it is therefore impossible to predict whether dung beetle larvae survive flooding, or how survival may vary by species. Thus, without clear measurements of the length of inundation within each sample site or knowledge of the ability of each dung beetle species to survive inundation, it is difficult to determine the effect of seasonal flooding on this community and the subsequent ecosystem services. It is possible however, that seasonal flooding may lead to an annual depression in dung beetle abundances during months when inundation occurs.

In contrast to the effects of seasonal flooding on dung beetle abundances, a lack of precipitation may have also had impacts on dung beetle abundances. Dung beetles were observed to have peaks in abundance both before the experiment (July) and after (December) (Figure 26). However, in the months immediately preceding the experiment, rainfall in the area was unusually low with no precipitation occurring from August-October. Fincher (1973) observed that soil desiccation negatively affected brood ball survival in sandy soils, which are the dominant soil-type at the study site. Thus, it is possible that the dry conditions preceding the execution of the experiment led to a reduction in soil moisture and decreased dung beetle larvae survival. While beetles were not trapped during the experiment in order to prevent possible interference with the experiment, it is likely that a drought-induced population depression would have persisted until rain returned in November. Therefore, the experimental outcome may have been dampened due to low dung beetle abundances during the experiment as a result of the dichotomous union of early summer seasonal flooding and a late summer dry-spell.

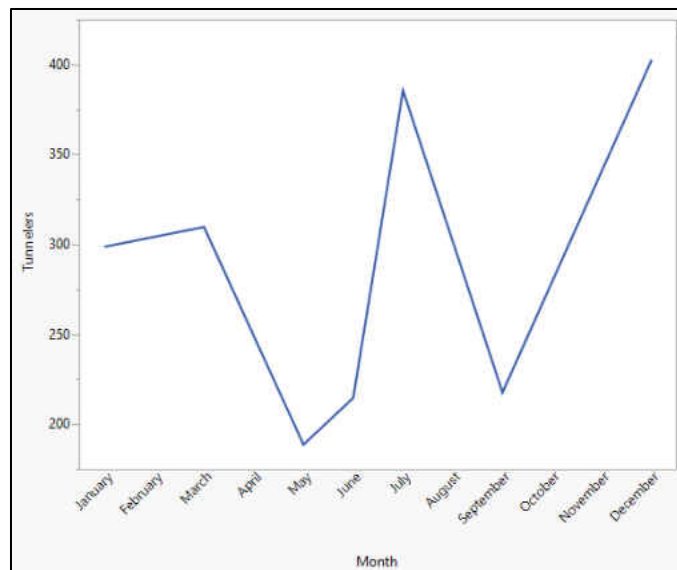
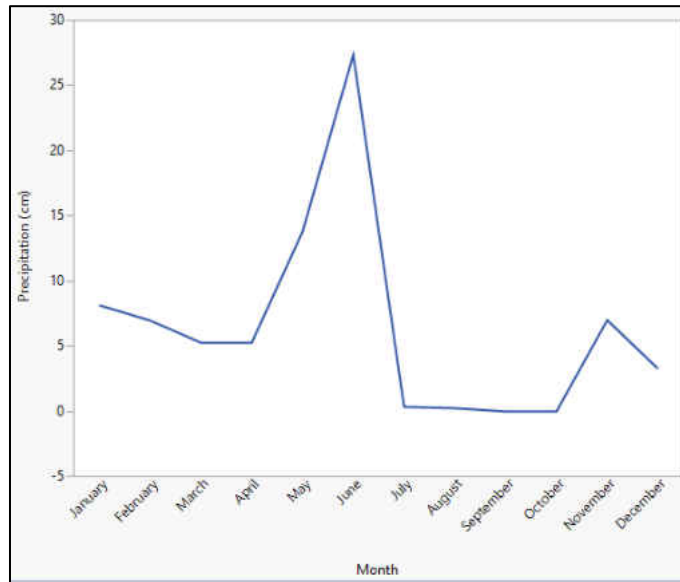


Figure 26. Top: Precipitation records by month for 2014. Bottom: Total tunneler abundances from all trapping sites.

Conclusion

Although the dung beetle treatment was found to reduce parasite survival, there was not enough evidence to conclude that fire ants impacted parasite abundance or dung beetle activity. The mechanism through which dung beetles reduced parasite survival does not appear to be related to the burial or removal of dung. Future research could investigate the mechanism for parasite suppression further through experimentally testing the separate impacts of feeding activity vs. movement and subsequent pat desiccation. Continued research into how dung beetles provide the ecosystem service of parasite suppression may also elucidate how individual beetle species or reproductive groups vary in their contributions.

CHAPTER 5: CONCLUDING REMARKS

Grazing animals in Florida deposit over 20 million metric tons of dung per year, making dung a significant non-point source of pollution (extrapolated from Fincher, 1981). Degradation of this dung occurs naturally, primarily due to a diverse group of beetles (Order Coleoptera) primarily in the families Scarabaeidae and Geotrupidae (hereafter dung beetles). Dung that is not degraded may be leached into water bodies and provides an incubation site for the pests and parasites of both humans and livestock. Thus, dung beetles provide an ecosystem service by increasing the rate of decomposition of dung in pasture ecosystems. The optimization of the ecosystem services provided by dung beetles is a priority for not only the protection of terrestrial and aquatic biota, but the cattle industry itself. The non-native fire ant *Solenopsis invicta* has been observed to frequently utilize dung as a food resource and may be predated or competing with dung beetles. Alternatively, there may be little or no interaction, thus it is important that we better understand the dynamics of the interactions of these arthropods in dung. My research will utilize semi-natural and intensively managed pastures to determine how management driven differences in habitat alter dung beetle assemblages. I will also experimentally evaluate how pasture type and the presence or absence of the non-native *Solenopsis invicta* influence dung beetle community assembly and the ecosystem services of dung degradation, parasite suppression and pest suppression.

APPENDIX: ADDITIONAL TABLES

Table 16: Description of data collected on each plot within each pasture.

Pasture	Plot	RESPONSE	MANAGEMENT	ENVIRONMENTAL VARIABLES				GEOGRAPHIC VARIABLES			HISTORIC MGMT VARIABLES			
		VARIABLE	INTENSITY	Soil Moisture (%)	Soil Temp. (°C)	Total Cow Pies	Veg Height (cm)	Road	Wetland	Ditch	Time Since	Time	Time	Time
Number of Mounds	Pasture Type	Disturbance	Since Soil								Since Chop	Since Aeration	Since Mow or Dragging	
Bull Field	1	6	Intensive	26.5	23.35	9	3.25	11.49	109.23	22.29	1	1	2	2
Bull Field	2	13	Intensive	19.25	24	5	8	7.74	133.39	19.48	1	1	2	2
Bull Field	3	5	Intensive	24.65	22.35	10	10.5	279.65	102.24	18.01	1	1	2	2
Bull Field	4	11	Intensive	20.6	22.55	14	7.75	114.92	87.76	21.63	1	1	2	2
Bull Field	5	0	Intensive	20.6	21.95	7	12	133.86	127.74	36.18	1	1	2	2
Ceiling	6	2	Intensive	11.95	29.5	3	17.75	29.25	44.57	5.57	1	7	2	5
Ceiling	7	2	Intensive	11.65	29.5	1	7.75	191.90	26.19	6.14	1	7	2	5
Ceiling	8	1	Intensive	27.45	29.5	1	6	58.70	102.98	25.78	1	7	2	5
East Marsh	9	1	Semi-native	18.7	29.75	2	33.25	12.90	131.02	303.01	1	2	6	0
East Marsh	10	2	Semi-native	19.65	26.15	8	16.75	16.62	60.35	79.75	1	2	6	0
East Marsh	11	8	Semi-native	17.65	26.5	9	15.5	15.71	55.46	24.80	1	2	6	0
East Marsh	12	2	Semi-native	14.7	26.35	0	43	126.08	67.51	123.96	1	2	6	0
East Marsh	13	2	Semi-native	28.4	26.75	5	14.75	113.77	54.12	46.96	1	2	6	0
East Marsh	14	2	Semi-native	25.25	25.85	10	13.75	120.33	159.79	235.92	1	2	6	0
East Marsh	15	3	Semi-native	22.2	29.5	1	38.5	118.14	91.16	346.74	1	2	6	0
East Marsh	17	7	Semi-native	19.7	25.65	9	44.5	103.02	104.21	163.82	1	2	6	0
East Marsh	18	3	Semi-native	20.55	24.25	2	8.75	26.74	33.28	18.01	1	2	1	0
East Marsh	19	2	Semi-native	16.55	23.6	3	16.75	21.71	78.62	14.82	1	2	1	0
East Marsh	20	3	Semi-native	17.6	23.15	8	19.25	197.93	24.81	18.25	1	2	1	0
East Marsh	21	6	Semi-native	15.6	27	6	35.75	179.16	18.44	158.39	1	2	1	0
East Marsh	22	1	Semi-native	18.05	22.85	2	15	222.80	46.65	17.98	1	2	1	0
East Marsh	23	5	Semi-native	27.65	25.75	3	14.5	115.50	44.14	5.43	1	2	1	0

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