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Biotic and Abiotic Characteristics Influencing Nest Location and Trophic Relationships of the Fungus-Growing Ant *Trachymyrmex septentrionalis* (Formicidae: *Attini*)

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BIOTIC AND ABIOTIC CHARACTERISTICS INFLUENCING NEST LOCATION AND TROPHIC RELATIONSHIPS OF THE FUNGUS-GROWING ANT *TRACHYMYRMEX SEPTENTRIONALIS* (FORMICIDAE: *ATTINI*)

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ABSTRACT

BIOTIC AND ABIOTIC CHARACTERISTICS INFLUENCING NEST LOCATION AND TROPHIC RELATIONSHIPS OF THE FUNGUS-GROWING ANT *TRACHYMYRMEX SEPTENTRIONALIS* (FORMICIDAE: *ATTINI*)

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Trachymyrmex septentrionalis represents the only species of fungus-growing ant in the southeastern United States. *T. septentrionalis* cultivates a symbiotic fungus on which the ants feed. Worker ants collect plant and animal debris to feed the fungus. These ants are common in Florida, but increasingly scarce as they near the northern extent of their range in New Jersey. Colonies of *T. septentrionalis* occur in patches throughout Blackwater Ecological Preserve, a longleaf pine forest in southeastern Virginia. Blackwater Ecologic Preserve is a 129 hectare tract of land, located in Isle of Wight county, that is undergoing a regimen of controlled burning to increase the population of longleaf pines (*Pinus palustris*).

Over an 8 year period, multiple abiotic and biotic factors were studied to better understand what conditions determine the distribution of *T. septentrionalis* colonies within an ecosystem and how those factors may affect the ability of individual colonies to collect sufficient substrate to sustain the fungal symbiont and feed the colony. Soil temperature (23.5 vs. 25.1°C), air temperature (22.3 vs. 26.4°C), and light intensity (302.8 vs. 595.2 lumens) were all found to be significantly higher around inactive nests. Plant cover, which would affect light intensity around the nests, was significantly higher above active nests (71.1%) than over inactive nests (57.9%). Soil samples from *T.*

septentrionalis populated and adjacent, unpopulated sites resulted in soil moisture being significantly lower in the upper 60 cm of soil in populated versus unpopulated areas. No consistent differences were found for soil organic matter, cation exchange capacity, or pH between populated and unpopulated sites. Colony densities, calculated for each nesting site, ranged from 0.072 to 0.145 nests/m^2, and these densities can be correlated to soil and air temperatures, light intensity, and vegetation cover.

In an attempt to understand the patchy distribution of *T. septentrionalis* in Blackwater Ecologic Preserve, the composition of ant and plant communities in areas with and without *T. septentrionalis* nests were analyzed. A total of 34 ant species was collected, with 27 species from sites with T. septentrionalis and 30 from those without. Only *Aphaenogaster treatae* displayed significantly different numbers between sites, but there were numerous species that occurred only in one or the other. A total of 15 plant species were identified in areas populated by *T. septentrionalis*, but 22 were identified in unpopulated areas. As with the ant community, numerous species could be located in one site but not the other.

Just as air temperatures affect the overall activity of *T. septentrionalis* colonies, they also influence foraging rates. Field preference of forage falls into four categories (Berry, *Pteridium aquilinum*, *Quercus* sp., Unknown Materials). Carbon and nitrogen concentrations increase as the elements are tracked from the substrate to the fungus then to the ants themselves. During a season of activity, a nest of 356 workers, alates, and pupae is expected to capture approximately 29.58 kcal/m^2 of energy through substrate collection. From this total, the *T. septentrionalis*/fungus symbiont is estimated to assimilate between 11.1 and 12.79 kcal/nest/yr.

T. septentrionalis appears to be highly sensitive to environmental conditions such as temperatue and light intensity both for daily activities and the foundation of new colonies. Soil moisture may also be considered as an important factor, either directly as it influences chamber excavation and stability or indirectly by determining vegetative cover. Overall, ant and plant communities did not affect the presence or absence of *T. septentrionalis*. *T. septentrionalis* acquires limited nutrients and energy from the surrounding ecosystem, this may be due to an inability to exploit the environment or the higher energetic demands required to acquire richer sources of nutrients and energy by leaf cutting.

Proverbs 6:6-8
Go to the ant, thou sluggard; consider her ways, and be wise:
Which having no guide, overseer, or ruler,
Provideth her meat in the summer, and gathereth her food in the harvest.

Psalms 19:1-4
The heavens declare the glory of God; and the firmament sheweth his handywork.
Day unto day uttereth speech, and night unto night sheweth knowledge.
There is no speech nor language, where their voice is not heard.
Their line is gone out through all the earth, and their words to the end of the world.

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CHAPTER I

INTRODUCTION

Energy has long been used as the defining characteristic for community productivity (Odum 1959). However, in recent years, the effect of energetics on every aspect of ecology has produced increased interest in the Metabolic Theory of Ecology, which states that metabolic rate has a direct influence on ecological processes at every level, including life histories and population dynamics (Brown *et al.* 2004). The dynamic structure of a population is determined by intra- and inter-specific competition (Culver 1974, De Vita 1979, Vepsalainen and Savolainen 1990, Gordon and Kulig 1996), nutrient/food supply (Howard 1987, Howard 1988, Johnson 1991), and abiotic conditions (Hodgson 1955, Lewis *et al.* 1974a-b, Levings 1983), but the driving force behind each of these factors is energy (Engelmann 1961, Golley and Gentry 1964, Menhinick 1967, Lugo *et al.* 1973, Deslippe and Salovainen 1995). Competition is influenced by how much energy is expended in a confrontation or the avoidance of confrontation (De Vita 1979, Harrison and Gentry 1981, Vepsalainen and Savolainen 1990, Gordon and Kulig 1996). The quality of nutrition for a population is a key indicator of the energy pool available for usage (Humphreys 1981, Huey and Pianka 1981, Halaj and Wise 2001), and the abiotic conditions control the amount of energy that is exerted during the activity of the organism, as well as provide the foundation of the nutrient supply. This study will focus on the life cycle and activity pattern of the fungus-growing ant *Trachymyrmex septentrionalis*, as defined by the energy flow through the system.

This dissertation follows the model format of *Ecology*.

Evolutionary History

Fungus-growing ants (*Attini*) originated approximately 50 million years ago, and they are found only in New World ecosystems (Wilson 1971). Fungus-growing ants are unique because they are able to produce their own food through agriculture. The most recognizable of these insects are the leaf-cutter ants that use fresh cut leaf fragments as the substrate on which their fungal farms subsist. The genus *Trachymyrmex* is the most basal member of the higher attines and can be dated at a minimum of 20 million years old (Schultz and Meier 1995, Wetterer *et al.* 1998). The majority of the approximately 200 species of *Attini* exist in the tropical forests of Central and South America between the latitudes of 40°N and 40°S (Lenczewski 1985); however, one species, *Trachymyrmex septentrionalis*, ranges into the central Atlantic coastal region of the United States, where a temperate environment is maintained by the Gulf Stream.

Five explanations have been proposed for the origin of fungus-ant symbiotic relationship. Wheeler (1907) states that the modern fungus-growing ant evolved from growth of fungus on ant feces deposited on nest midden piles. Another possibility is the ants evolved from predaceous ants cohabitating rotting logs with fungi, which used the insects' feces as substrate (Forel 1902). von Ihering (1894) introduced two hypotheses: that the symbiosis originated from fungus growing on the stores of seed-harvesting ants, or that the relationship stemmed from fungus growing on the corpses of arthropods that were deposited near nest entrances. The most recent argument is the ant-fungus relationship stems from the gradual development of symbiosis between ants and a mycorrhyzal fungus (Garling 1979). Each of the above theoretical origins assumes that the ant initiated the evolution of the relationship, but Bailey (1920) offered the

alternative explanation that fungus-growing began due to the use of ants as dispersal systems for fungi. The most widely supported of the hypotheses is that of Garling (1979) because leaf-cutting ant colonies often establish their fungus garden on a single rootlet protruding through the garden chamber excavated by a founding queen (Weber 1966; Cole 1939). However, more recent analysis of the evidence may be shifting support to a fungus-initiated origin of the fungus-growing ant symbiosis (Mueller *et al.* 2001).

Life History

There are currently 40 known species of *Trachymyrmex* in the New World (Urbani 1980), but *Trachymyrmex septentrionalis* represents the only species of the fungus-growing ants common in the southeastern United States. *T. septentrionalis* range from Florida to their northern limit of Long Island, New York where they occupy ecosystems characterized by pine forest and sandy soils (Weber 1972). *T. septentrionalis* is distributed along the eastern coast of the United States in patches because of selective habitat restrictions, such as those found in Blackwater Ecological Preserve (BEP; described below).

The mating flight of ants in the genus *Trachymyrmex* generally occurs once a year per nest during the early portion of the summer (Cole 1939, Weber 1956a, Weber 1967, Lenczewski 1985). After mating, the female loses her wings and constructs a chamber in the second layer of soil. Within this chamber, the female deposits a clonal body of the fungus garden that she removed from her original colony and carried in her mouth during mating (Diamond 1998). The female lays her first brood of eggs directly on the fungus garden and tends the fungus herself by supplying feces and saliva as

substrate before foraging for vegetational substrate until her first offspring mature and take over the task of gardening (Diamond 1998 and Mueller *et al.* 1998). Founding queens of *T. septentrionalis* are not grouped with the claustral queens of *Atta* sp. because they lack the initial energy reserves, in the form of stored fat, to maintain the fungus garden without foraging for additional substrate; *Atta* sp. queens have 40%, or more, stored fat, while *T. septentrionalis* have approximately 25% (Keller and Passera 1989, McInnes and Tschinkel 1995, Tschinkel 1996, Seal and Tschinkel 2007).

T. septentrionalis colonies maintain small but constant colony sizes, approximately 100-200 members. During the first year of colonization, a new colony attains a member number and fungus garden volume close to that of a mature colony, and the number of alate reproductives produced is very similar to that of a mature colony (Lenczewski 1985). However, most new colonies are not likely to last for a year. Most of the information on queen mortality comes from studies of *Atta* species, another member of the Attini. The mortality rate for new *Atta* queens is 97.5% within the first 100 days of foundation, and 99.95% of colonies cannot sustain themselves beyond the first 15 months of establishment (Autuori 1941). Hernandez *et al.* (1999) found a mortality rate of 45% during the ninth month of their three-year study on *Atta laevigata*. This rate eventually fell to 25% in the third year, but there is no indication of the rate during the first eight months of the study. Also, increased densities of nests can attract larger numbers of predators, or high density areas may outstrip available resources (Lenczewski 1985).

Within the work structure of the colony, there is little or no division of labor, and all members, except the queen and the alates, are monomorphic or weakly polymorphic

(Beshers 1993). This physical characteristic is a major difference distinguishing *T. septentrionalis* from the leaf-cutters, *Atta* and *Acromyrmex*, which are strongly polymorphic. Unlike the more prodigious leaf-cutting ants, *T. septentrionalis* does not use freshly cut materials as the primary substrate of the fungal garden but collects plant and animal detritus (Cole 1939). The gathering of substrate materials by *T. septentrionalis* is performed by small foragers of uniform size (Beshers and Traniello 1996). Since *T. septentrionalis* does not harvest fresh materials, there is no need for worker size specialization within the colony.

Environmental Factors Affecting Activity Patterns

Very few data have been published concerning factors affecting the activity patterns of *Trachymyrmex* (Weber 1956a-b, Cole 1939). Changes in temperature have shown strong correlations with alterations in metabolism in many species. Gillooly *et al.* (2001, 2002) and Savage *et al.* (2004) have demonstrated the significant relationship between temperature and community structure, interspecific relationships, population and individual growth, and life history. *T. septentrionalis* is active only within a limited temperature range. Weber (1956a) was able to maintain active colonies of *T. septentrionalis* in his laboratory within the temperature range of 18-24°C. Numerous observations have also been made of the limited activity of attines during the middle part of the day when subject to direct sunlight (Hodgson 1955, Lewis *et al.* 1974a-b). However, there are some questions as to whether this is more of a reaction to the increased temperatures during those times of the day than to light intensity (Weber 1967, Cole 1939). Weber's (1956a-b) observations of *T. septentrionalis* nests in Florida demonstrate an active temperature range from 21.6 to 30 °C. Cole (1939) never

quantified his findings of observed *T. septentrionalis* activity, but noted that *T. septentrionalis* generally exhibit nocturnal activity during the summer except on cool days. Additional studies by Box (1960) on a seed harvester ant (*Pogonomyrmex barbatus*) and Hodgson (1955) and Lewis *et al.* (1974a-b) on *Atta cephalotes*, and Bernstein (1979) on multiple species of ants all indicate a correlation between temperature and activity. Kaspari (2001) found that populations of *T. septentrionalis* are more significantly influenced by changes in temperature than by changes in the primary productivity of their environment.

Light intensity has also been indicated as an important factor in determining ant activity, although none of the following studies controlled for temperature. Weber (1967) observed that *Trachymyrmex jamaicensis* was active only when the sun did not shine directly on the nest. Foragers from *Atta cephalotes* begin above ground activity when light intensity reaches 3.2 lux (Hodgson 1955). However, Lewis *et al.* (1974a-b) found that foraging could occur during a range of 0.05 – 100,000 lux in their study of the diel foraging pattern of *Atta cephalotes*. Despite this wide range of light intensity measurements, Lewis *et al.* (1974a-b) found that the correlation between ant activity and light intensity is significant.

The intensity of light at the nest entrances is influenced by the density of cover materials surrounding the ant populations. Blanton and Ewel (1985) concluded that *Atta cephalotes* prefer more disturbed, open areas. *T. septentrionalis* has also been observed to inhabit primarily disturbance mediated, open environments (Weber 1956a and Lenczewski 1985). Two advantages for *T. septentrionalis* living in a disturbed, open

area may be the subsequent increase in available light and a more diverse array of plants from which substrate may be collected.

The patchiness of an environment is influenced by climate, soil, and disturbance (Wiens 1976). Variation of microclimate affects growing conditions for vegetation, resulting in changes in community structure across localized areas (Hinds 1975). Changes in soil texture can also directly affect the ability of vegetation to germinate producing variation in community structure throughout a landscape. When alterations to microclimate and soil texture are combined, hydrologic conditions are affected which influence absorbance, or runoff, of water by an area, further driving the vegetation of an environment towards a patchy mosaic (Zedler and Zedler 1969). Open patches are generally located in areas created by moderate to high disturbances such as fire and windfall. Leaf-cutting ants have demonstrated a preference for these types of open, patchy spaces because of the high plant diversity and new growth (Blanton and Ewel 1985).

The chemistry and characteristics of the soil in which *T. septentrionalis* make their nests were also examined in the present study. The most detailed study of the soil characteristics of preferred nesting sites of *T. septentrionalis* were performed by Cole (1939), who characterized the dominant texture and relative moisture at successive depths. *T. septentrionalis* habitation sites are typified by three distinctive soil layers (Cole 1939). The upper layer is a loose, dry sand extending to a depth of approximately 11 cm. An intermediate layer consists of dry, packed sand that extends 16 cm deeper through the soil. The lowest layer is a moist, packed sand extending to the water table. Each chamber that is occupied by a fungus garden contains a small, individual rootlet

extending from one side of the chamber to the other (Weber 1966, Cole 1939). Quantifying and categorizing the soils inhabited by *T. septentrionalis* should provide more detailed insight into the characteristics that govern the establishment of new or maintenance of old colonies.

Colony Dispersion

The distribution of ant species is affected by factors occurring at two different levels. Theunis *et al.* (2005) studied the community structure of litter-dwelling ants in Argentina. Their findings indicate that large scale distribution (>50 m) is primarily a factor of environmental conditions such as temperature, moisture, and canopy cover. At a smaller scale (<10 m), colony distribution is attributed to intraspecific competition.

Territories persist in either circular or hexagonal patterns (Covich 1976). Circular patterns are most efficient for organisms with central storage sites because energy loss could be minimized through the reduction of travel time and exposure to predation (Smith 1968). However, this pattern of territory establishment does not take into account the free spaces developed between bordering circles of influence where no organism establishes a territory. These free spaces have resulted in the consideration that hexagonal patterns would be the most economically feasible patterns for territory establishment (Morrill 1970 and Isard *et al.* 1972). Hexagonal patterns minimize travel time, and associated risks, just as with circular territories, while also maximizing the usage of the surrounding resources (Covich 1976). Populations established in areas with homogeneous distribution of resources produce evenly spaced hexagonal territories. As resources become more patchy in distribution, territorial boundaries will shift accordingly, producing a mosaic of territory sizes and shapes (Covich 1976).

Patchy environments are colonized by populations employing coarse-grained dispersal strategies (Bryant 1973). In this structure, the environment is relatively stable, but there is obvious variation in patch quality, and tendencies towards large population conglomerations are not uncommon (Wiens 1973). Many species of insect can be classified as course-grained, and representative populations of these insects have well-developed dispersal mechanisms (Southwood 1962). The ability of populations to colonize in patches across an area may reduce the risk of widespread extirpation due to spontaneous natural events (den Boer 1968).

Colonies of *Trachymyrmex septentrionalis* in Florida have greater success in more disturbed forested areas, along roadsides, and in small forest clearings than colonies established in less disturbed areas (Lenczewski 1985). *T. septentrionalis* colonies are significantly affected by changes in ground temperature as influenced by the level of vegetation cover (Seal and Tschinkel 2006). Populations of *T. septentrionalis* experience higher colony densities and greater productivity in open, warmer areas than in cooler, shaded areas; however, establishment of colonies is not only a function of abiotic conditions, but may also be a function of intra- and inter-specific competition. Brian (1956) suggests that initial colonization is determined by abiotic site characteristics, but after establishment of the colonies, distribution is influenced by territorial behavior as determined by food supply.

A conservative mortality rate for *T. septentrionalis* colonies in Florida was calculated at 56% for newly founded colonies and 72.5% for colonies over one year of age (Lenczewski 1985). Several possible explanations have been offered for these low survival rates. A study of *Atta capiguara* found that conspecific workers from

neighboring colonies often executed new queens as they attempted to burrow into the soil (Fowler *et al.* 1984). Gordon and Kulig (1996) found that the age and distance of neighboring colonies had a significant influence on the survival of new colonies for the harvester ant, *Pogonomyrmex barbatus*. One-year old nests were more likely to be established and survive when they were next to two or three year old nests than when their neighbors had been established for five years or longer. This correlation may not be as important for *T. septentrionalis* because the average colonial lifespan may be as low as 1.7 years in nature (Lenczewski 1985); however, Weber (1956a) was able to sustain queens in captivity for up to five years. Other possible explanations suggested by Lenczewski (1985) are that high density areas of *T. septentrionalis* may attract increasing numbers of predators that could decrease the survival of new colonies, or that substrate impoverishment by already established colonies could reduce survival of new colonies, thus severely retarding their nutrient and energy intake during the critical stages of colony establishment and development.

Lenczewski (1985) found colony densities ranging from 0.3 to 0.14 nests/m^2 for *T. septentrionalis*. Colony densities for other species of ants appear to be primarily determined by food availability (Brian 1956, Smallwood and Culver 1979). Obviously, the amount of available food is significant because fewer nutrients and less energy would be supplied to the fungus farms and the reproductive ability of the nests would be deleteriously affected. Nests located in high-density areas have been shown to be significantly smaller, based on numbers of individuals, than colonies in lower density areas (Lenczewski 1985).

No data currently exist concerning *T. septentrionalis* and inter-specific competition. Lenczewski (1985) did not observe any direct competition over the course of her study in Florida. Despite the lack of literature, numerous sources (see Connell 1983, Schoener 1983) indicate the manifestation of competition when two or more species are dependant on the same resource pool.

Lenczewski (1985) observed that established *T. septentrionalis* nests periodically move their nest entrances. Lenczewski (1985) states that *T. septentrionalis* can move their nest entrances during successive years, and assumes that any new nests located within 25 cm of old nest entrances in following year of study were the same nests. No evidence is provided for this assumption. However, nest migration has been noted in other studies. Wilson (1971) observed nest migrations of *Atta* and *Pogonomyrmex* after extreme nest disturbances, and *Aphaenogaster rudis* and *Tapinoma sessile* migrate due to predation and parasitism (Smallwood and Culver 1979).

Forage Behavior

T. septentrionalis forages randomly, with no set trails, and generally as individuals without large search parties (Cole 1939, Weber 1958). *Trachymyrmex septentrionalis* workers are extremely timid and instead of struggling to escape, they will often play dead when handled roughly (Weber 1966). While *T. septentrionalis* may occasionally forage from a trail, the majority of foraging is performed on an individual basis (Weber 1956a), which contrasts to the massive foraging campaign undertaken by the tropical leaf-cutter ants. Foraging *Atta colombica* show no dedication to a particular food type, and an individual may collect substrate from a variety of sources during any period of activity (Shepherd 1985). In *Trachymyrmex turrifex*, the majority (over 50%)

of substrate materials returned to a colony is of plant origin, and approximately 20% of the material is detrital material from other insects or animals (Waller 1989). Quinlan and Cherrett (1979) observed that in *Atta* colonies the fungus garden provides the sole food source for colony larvae, but that 90% of the adults' nutrient intake was from leaf fluids. This foraging pattern is similar to that noted by Bernstein (1979) for two species of *Pogonomyrmex* and a species of *Veromessor* when foraging resources were plentiful. For each of these three species, distance of foraging activity from the nests affected the overall abundance of colonies in an area (smaller foraging areas increased nest density). Also, the ants were able to reduce intra-specific competition by regular spacing of colonies.

The flow of energy into and through a system is the most important factor influencing growth, development, and success of that system. *Trachymyrmex septentrionalis* is considered to be a fungivore (Bailey 1920). Carroll and Janzen (1973) divided ant foraging strategies into five categories: hunting, raiding, seed collecting, scavenging, and harvesting. While higher attines are classified in the harvesting category due to their territoriality and selective foraging patterns (Cherrett 1972, Rockwood 1972), the lower Attines, including *Trachymyrmex*, are subjugated to the scavenging category. Scavengers are defined as ants specializing on unpredictable food sources with colonies occurring in centralized patches. The effects of unpredictable and low quality food sources tend to produce smaller individual workers and smaller colony sizes as a means of maximizing cost/benefit economies.

T. septentrionalis forage a greater diversity of materials for their fungus gardens than the true leaf-cutting ants. While *T. septentrionalis* use some fresh cut plant

materials in their gardens, they primarily forage for caterpillar frass and detrital plant matter (Cole 1939, Weber 1956a). Freeland and Janzen (1974) provide seven principles for efficient herbivory. Not all of these principles can be applied directly to *T. septentrionalis* since they collect primarily detrital material; however, some substrate is gathered as fresh material. Therefore, many of these principles can be altered in their application to *T. septentrionalis* since any selection of fresh materials will increase the exposure of the fungal garden to harmful secondary chemicals (Hubbell *et al.* 1984). Herbivores should be cautious of new foods. A rapid dissemination plan must be developed to accept or reject a given food. They must have the ability to discriminate plants with the most valuable nutrient and energy content. A diverse pool of acceptable plants should be established, and sampling of new plants should be continuous. All preferred plants should be used for as long as possible. Plants should have low amounts of toxic secondary chemicals. And lastly, a searching strategy that minimizes the number of plants and amount of plant material required for self-preservation should be developed.

Several of these principles have been demonstrated in the literature for species of *Atta*. Most plants are acceptable at some stage of their life, and they change from palatable to unpalatable during the season (Rockwood 1972). *A. cephalotes* samples and attacks a wide range of host plants, while focusing most of their efforts on a select few (Mulkern 1967, Cherrett 1968). The ants use physical and chemical properties of the leaves to determine acceptability. Increases in leaf toughness deter attack by *A. cephalotes* and *A. texana* (Cherrett 1972, Waller 1982), thus explaining the tendency of leaf-cutters to attack young, thinner leaves (Fennah 1950, Cherrett 1972). High water

content has also been demonstrated to affect leaf preference (Cherret 1972, Bowers and Porter 1981). Howard (1987, 1988) found a high correlation between leaf palatability and secondary chemistry. Leaves with high levels of hydrolyzable tannins and proteins are highly palatable, while those with high levels of nonpolar extracts deter selection. In a study of 10 ant species, Kay (2002) found that the preferred substrate used by *T. arizonensis* contains the highest protein concentration, and lowest carbon to protein ratio, of any materials foraged by the ant subjects.

Since harvested materials are used as substrate for the fungus gardens of the ants, and the fungus is the sole source of nutrition for the ant larvae, then the nutritional needs of the fungus are the determining factors for what plant materials are selected for harvest (Quinlan and Cherrett 1979). However, the fungus gardens of *Atta* do not appear to provide all the necessary dietary components required for survival (Martin *et al.* 1969, Mueller *et al.* 2001). This indicates that the total level of dietary component (carbohydrate, lipid, and protein content) may not be as much of a determining factor for nutritional value as the ratio of the dietary components to one another (Mueller *et al.* 2001).

Energetics

Atta colombica has a significant impact on energy flow through the tropical ecosystem. These leaf-cutting ants introduce foraging material to their nests with an energy value of approximately 126.1 Kcal/m^2/yr. They also increase net production of the forest by 657.0 Kcal/m^2/yr (Lugo *et al.* 1973). *T. septentrionalis* would not be expected to consume such large energy resources due to the smaller size of the colonies (Lugo *et al.* 1973 and Lenczewski 1985) and lower productivity of the ecosystem

[tropical forest 3250-3700 g/m^2/yr (Rodin and Bazilevic 1966) vs. pine-oak forest 1000-1600 g/m^2/yr (Whittaker 1966)]. However, similar types of foraging ant communities (*Dorymyrmex*, *Pogonomyrmex*, and *Trachymyrmex*) have demonstrated input values ranging from 14 Kcal/m^2/yr to over 56 Kcal/m^2/yr (Golley and Gentry 1964, Menhinick 1967).

This study tested the following hypotheses:

1) Mechanisms determining population densities: Site-specific physical factors (cover density, light intensity, soil composition) have a greater influence on population densities than interspecific competition. This hypothesis was tested by comparing plant and ant species counts and densities within *T. septentrionalis* populated and unpopulated areas.
2) Effect of substrate nutrient and energy content on foraging behavior:

   A) Nutrient and energy content of the various types of foraged material used as substrate will not vary significantly. This hypothesis was tested by comparing materials comprising the foraged substrate.

   B) Substrate will be selected by *T. septentrionalis* based on ease of collection and availability. This hypothesis was tested by comparing the composition of the materials foraged over the course of the active season compared with available forage material in the surrounding habitat.
3) Energy dynamics of the ant-fungus symbiosis: The fungus represents a necessary intermediary in the dietary chain of *T. septentrionalis*. By allowing the fungus garden to process the foraged substrate, *T. septentrionalis* is provided with a higher quality food item, producing a higher assimilation rate

than would be expected from direct consumption of foraged material. This hypothesis was tested by comparing the energy and nutrient content of freshly foraged materials and fungal samples.

CHAPTER II

MATERIALS AND METHODS

SITE DESCRIPTION

Blackwater Ecological Preserve (BEP) is located in Isle of Wight County, Virginia (Figure 1). This site is ecologically important as the northern most range of the long leaf pine (*Pinus palustrus*) and as one of only two reproducing stands of longleaf pine in Virginia (Frost 1982). The Old Dominion University Department of Biological Sciences currently owns and jointly manages the preserve with the Nature Conservancy and the Virginia Department of Conservation and Recreation. This team is attempting to return the preserve to a more natural state. BEP is approximately 119 hectares in size and is a fire dependent community. One aspect of management by ODU has been the employment of controlled burns to maintain vegetation diversity and facilitate the reestablishment of longleaf pine in the area. The first controlled burn of the site was performed in the winter of 1986 (Frost and Musselman 1987), and burns have been performed annually, or biannually, since the summer of 2001 (personal observation).

Over two years of preliminary research, seven separate colonized areas of *Trachymyrmex septentrionalis* were located in BEP. This study focuses on the four most stable colonized areas identified within the preserve. Three sites were located in burn unit 4, and a single site was located in burn unit 2 (Figure 1). At the onset of this study, units four and two were burned in February and July of 2001, respectively. The burning of these areas early in the study allowed colonized areas to be clearly defined in surface area and relationship to one another. Burn units containing populations were subjected to a total of nine controlled burns prior to the end of the study.

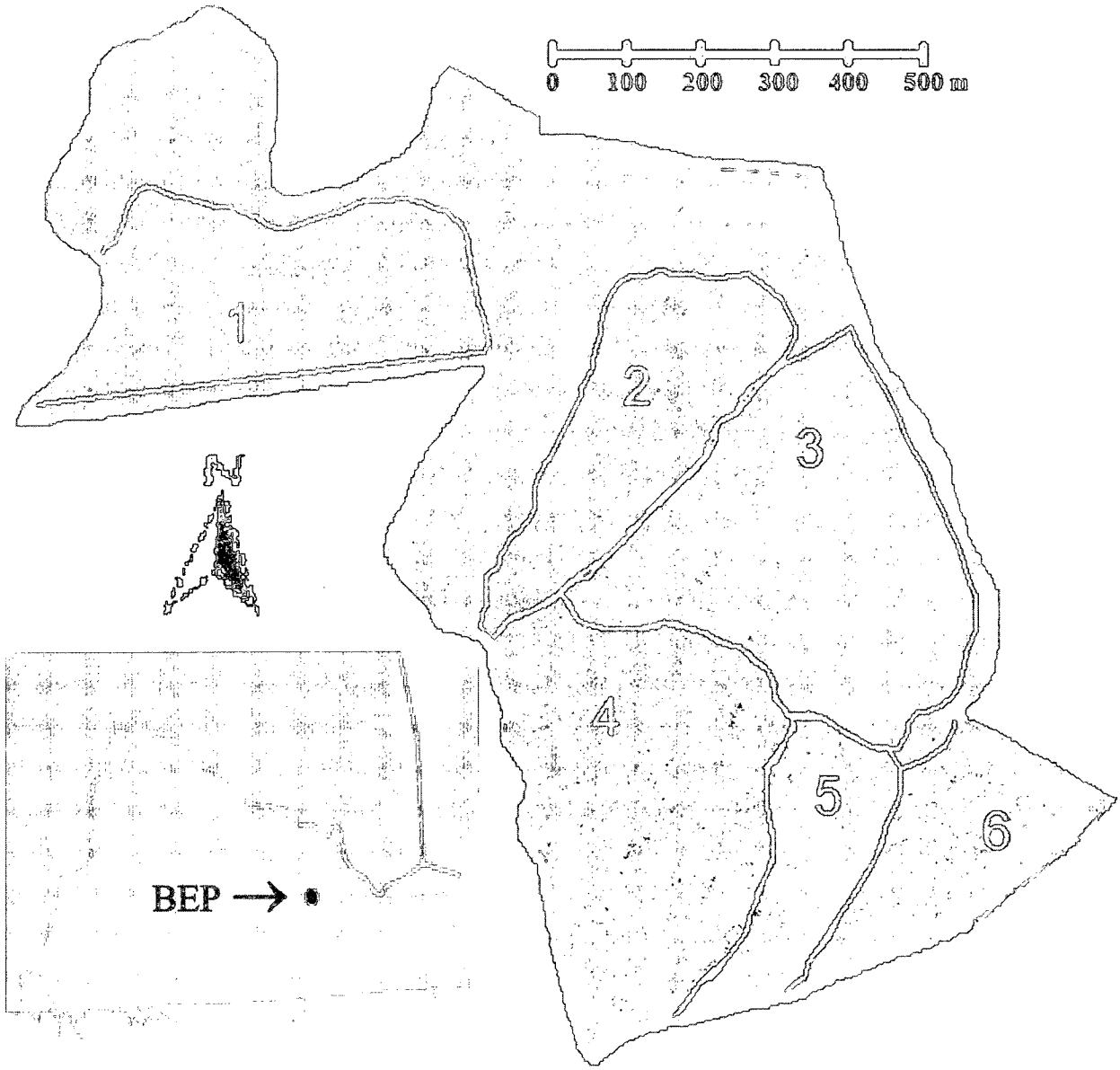

FIG. 1. Map of Blackwater Ecological Preserve. Numerals indicate designated controlled burn areas. All study areas populated by *T. septentrionalis* are located in areas two and four. (Map constructed by Darren Loomis)

TABLE 1. Locations and dates of controlled burns within BEP.
(Data compiled by Darren Loomis)

Burn Unit	Date of Burn
4	2/09/2001
2	5/31/2001
4	5/01/2003
2	4/22/2004
4	5/02/2005
2	5/05/2005
4	5/25/2006
2	3/23/2007
4	4/11/2007

The primary abiotic factors studied at BEP were air and soil temperatures, light intensity, vegetation cover density, and soil chemistry and characteristics. Data were collected from July-November 2000 and April-October 2001. During these time periods, collection occurred once a week. The possible effect of each of these factors on energy management by the ants was the focus of this study.

ABIOTIC CONDITION – ABOVE GROUND

Temperature

Temperature measurements for air and soil were performed on nests that demonstrated activity during the study. Air temperatures were taken with an indoor/outdoor thermometer (Fisher Scientific model 800) at a height of 10 – 20 cm above the respective nest entrance, while soil temperatures were made by inserting the probe to a depth of 2 – 3 cm at a distance of 8 – 10 cm from the nest entrance. Year 2000 data were collected on sampling days between 12:00 and 17:00 pm, while 2001 data were collected between 8:00 am and 12:00 pm with the exceptions of April 27 and

May 4, when measurements were made between 12:00 pm and 4:00 pm. All marked nests were used to collected data during each weekly visit.

Air Humidity

In conjunction with temperature measurements, atmospheric humidity levels were also measured, using the hygrometer function of the thermometer, above each nest at the same height as the air temperature measurements. Data were collected weekly from August-October 2001.

Light Intensity

Light intensity levels were taken using a photometer (LiCor Light Meter model LI-250). The light sensor was placed on the soil surface directly adjacent to each nest entrance (8 – 10 cm). The photometer automatically calculated 15 second averages. Data were collected weekly from April-October 2001 for every nest that had been identified in BEP.

Vegetation Cover

A spherical densiometer was used to determine individual vegetative cover values. Two separate cover readings were taken for each nest: ground level (5 – 10 cm) and chest level (~1.5 m). Total cover at both levels was calculated for each nest by averaging the readings taken from four directions located at 90° angles from each other. Data were collected weekly from April-October 2001.

ABIOTIC CONDITION – BELOW GROUND

Soil Samples

Soils directly adjacent to active nests and soils from nearby uncolonized areas of the preserve were compared using a variety of laboratory and field tests. A 2 cm x 30 cm corer (Oakfield Model K) was used to collect samples to a depth of 90 cm. Five cores were collected from three areas colonized with *T. septentrionalis* and three uncolonized areas, for a total of 30 cores. Two parallel 50 m transects were established in each of three areas with *T. septentrionalis* and three areas without *T. septentrionalis*, for a total of 12 transects. Cores were collected at 10 m intervals along these 50 m linear transects. These cores were also divided into upper (~1-30 cm), middle (~31-60 cm), and lower (~61-90 cm) soil layers. Prior to the collection of samples, all loose detrital material was removed from the soil surface. Cored soil samples collected during the first year were tested for water content, soil texture, cation exchange capacity (CEC), pH, and organic matter content. During the second year, no cores were made. Instead, field tests for pH and water content were performed during the second year. No significant difference was detected in preliminary studies between data from tests performed in the laboratory and data gathered directly in the field.

Water Content

Water content was determined for each core by oven drying samples at a temperature of 70°C for 48 hr (Thien and Graveel 1997). Samples of 5.0 g were placed in aluminum pans and placed in the oven. After 48 hr, the samples were removed, allowed to cool, and the soils were reweighed. Each of the three layers of each core was analyzed three times to insure consistency.

During the second year of the study, a field acidity and moisture meter (Kelway model HB-2) was used to collect data at each of the 30 core sites, 15 from areas with *T. septentrionalis* and 15 from areas without *T. septentrionalis*. All loose debris was removed from the soil surface, and the probe was inserted into the soil to a depth of 8-9 cm and allowed to stabilize for two minutes. Following stabilization, a % moisture range and an exact pH, could be read. The metal detection plates of the meter were wiped clean with a paper towel following each measurement.

Soil Texture

Soil texture was quantified by passing samples through a series of three mesh sieves (Hubbard model 3076). Soils were oven dried at 40°C for three days (Haney *et al.* 2004) before being placed in the sieve (Thien and Graveel 1997). The sieves separated the soil into three compartments representing large organics and rocks (> 2 mm), sand (2 mm - .06 mm), and silt and clay (< 0.06 mm).

Cation Exchange Capacity

Total cation exchange capacity (CEC) for the soil samples was determined colormetrically (Thien and Graveel 1997). Soil components that had been sieved were recombined with the exception of the large, organic materials. Samples of 1.0 g were first placed in a volumetric flask and mixed with 10 ml of 0.2 N copper acetate solution to replace the native soil cations with Cu^{2+}. Following the replacement of the native cations, the soil-solution mixtures were strained through filter paper lined funnels. After the solution had drained through the soil, the soil was rinsed with deionized water, and the resulting solution was discarded. 15 ml of 1.0 N ammonium acetate solution was added to each soil sample to replace the Cu^{2+} with NH_4^+ and rinse the Cu^{2+} from the soil

into solution. Deionized water was then leached through the soil until the total solution volume of the Cu^{2+} solution reached 20ml. The resulting solutions were then mixed with 5 ml of ammonium hydroxide to develop a blue, cupric color and prepare the sample to be analyzed using a spectrophotometer (Spectronic 20D). These data were referenced to a standard line (Figure 2), resulting in the measurement of the concentration of copper per gram of soil that could then be used to calculate CEC. Standard solutions were prepared by mixing volumes of copper acetate solution between 1.0 ml and 0.001 ml with 15 ml of ammonium hydroxide and enough deionized water to attain a volume of 20 ml. Copper concentrations for the standard curve ranged from 100% to 0.1% of the initial solution. The resulting curve produced an R^2 value of 0.9831.

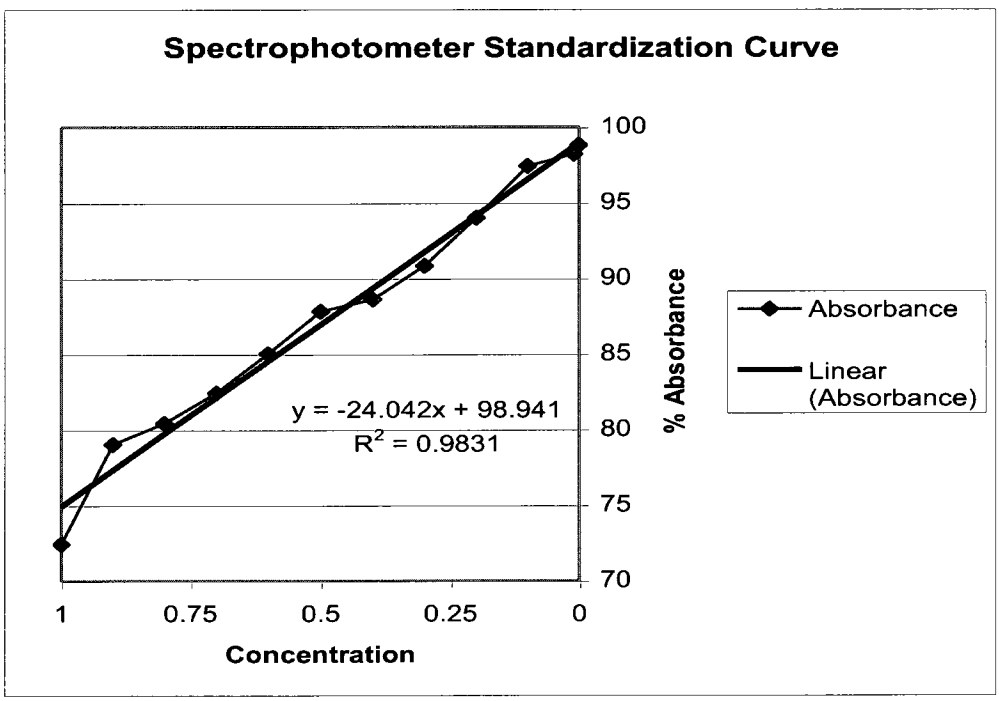

FIG. 2. Cu^{2+} concentration curve used for the analysis of spectrophotometric data. The X-axis identifies fractions of the original Cu^{2+} solution, and the Y-axis indicates % of light transmitted through the Cu^{2+} solutions.

pH

In 2003, a pH probe (Corning model 240) measured soil pH after soil and deionized water had been mixed at a 1:1 (w/v) ratio (Thien and Graveel 1997). 30 ml of deionized water were added to 30 g samples of soil and mixed thoroughly. The pH probe was then inserted and allowed to stabilize before the data were collected. Deionized water was used to rinse the pH probe between measurements. Field measurements of pH during the second year were performed using the methods described in the Water Content section above.

Organic Matter Composition

Organic matter content was measured by combustion of organic matter in an oven at approximately 275°C for 72 hr. This temperature was selected to minimize weight loss due to volatilization of inorganic compounds (Gray and Dighton 2006). Soil samples of 5.0 g were placed in aluminum pans and placed in a preheated oven for 72 hr. Samples were then allowed to air cool before being weighed. Any decrease between initial and final weight was classified as organic matter. Organic matter was measured for the same 30 core samples collected for analysis of water content and pH.

BIOTIC CONDITIONS

Nest Distribution

During the first four years of the study, all confirmed active nests within the preserve were marked and mapped. A string grid was used to map the nests for the first two years. A series of 5 m x 5 m quadrants was established, and X and Y coordinates were determined. In subsequent years, as larger populations were found, and nests spread beyond the boundaries of the initial grid network, coordinates were determined

through the application of Heron's formula (Tonnies and Lemke 1994). For this methodology, two landmarks (a and b) must be established and then the distance between those two landmarks, as well as the distance between each of those landmarks and each nest (c), was measured. Those measurements were used to solve the formula:

$$s = 1/2\ (a + b + c)$$

Where s is the semiperimeter of a triangle. Following the calculation of the value of s, the angle A of the triangle was then determined using the formula:

$$\cos A = [2s\ (s - a)/bc] - 1$$

Once the angle of A was established, the X and Y coordinates were calculated with the following formulas:

$$Y = \cos A * b$$

and

$$X = \sin A * b$$

In order to produce consistency between the population maps from year to year, all flags used to mark nests from the previous year were left in the field until marking began the next year. Any new nests located within 30 cm of a nest marked during the previous year were considered to be the same colony, and the older coordinates were used as benchmarks to reference the new coordinates to the recurring nests (Lenczewski 1985). Population borders were defined by the most direct lines drawn through the entrances of the perimeter colonies. Area was then calculated and used to determine population density.

Ant Community Structure

Ant community structure was determined for three areas populated by *T. septentrionalis* and three unpopulated areas. Two parallel 50 m transects were established in each area, three with *T. septentrionalis* and three without *T. septentrionalis*, for a total of 12 transects. Pitfall traps (16 mm x 100 mm glass test tubes) were partially filled with soapy water and sunk into the soil along each transect at 5 m increments, with 10 traps per transect (Agosti and Alonso 2000). Pitfall traps were left in place for 1 week before being collected. Traps were collected once during each of the following months of 2004: June, August, and October. After collection, the test tubes were returned to the lab where excess water was removed and replaced by 95% alcohol to permit storage for future identification. All ants were then identified using a dissecting microscope and multiple identification keys (Creighton 1950, Wilson 1955, Gregg 1958, Wheeler and Wheeler 1963, Wheeler and Wheeler 1977, Trager 1984, Wheeler and Wheeler 1986, Johnson 1988, Holldobler and Wilson 1990, Shattuck 1992 a-b, MacKay 1993, Bolton 1994).

Vegetation Analysis

Vegetation community analyses were performed on two different scales (Gleason 1920). The 50 m transects used for determining the ant community structure were also used for vegetation analysis. Trees and shrubs >1 m tall were identified in 10 m x 10 m quadrants. One randomly selected quadrant was chosen along each transect by drawing a number (1-5) from a pool. Once a site had been established, all large vegetation was identified and quantified in the field to the species level. Small vegetation (<1 m tall)

was identified in 1 m x 1 m plots established within the initial 10 m x 10 m quadrants. Three plots were randomly chosen within each quadrant by drawing numbers (0-9) from a pool for both the X and Y coordinates (Figure 3). The vegetation within each plot was identified and quantified in the field to the species level. Any vegetation that was not identified in the field was quantified, photographed, and a stem and leaf sample was returned to the lab to be identified. The second year of the study was performed in the same transect, but not the same 10 m^2, or 1 m^2, quadrants. The change of quadrants was dictated by the random drawing and not by choice.

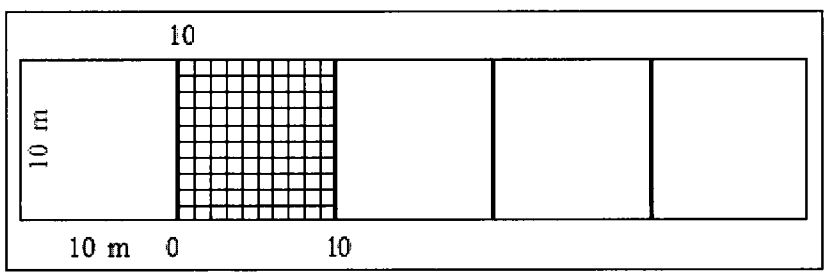

FIG. 3. Diagram of plotting methods used to identify vegetation species at the 10 m^2 and 1 m^2 level.

Foraging Analysis

Ant foraging data were collected in three segments (Lugo *et al.* 1973). The initial aspect of data collection was direct observation. Foraging colonies were observed for 1-minute intervals, and the number of foraged materials returned to the nest during that time was recorded. Attempts were also made during this period to identify the nature of the foraged materials. Following observation, foraged materials were collected for 1-minute. Forceps were used to remove the load of each forager that returned to the nest within a 1-minute time span. The foraged materials for each nest were individually packaged and stored in a laboratory freezer for later analysis. The final segment of data collection on the foraging activities of *T. septentrionalis* was to attempt to follow the foragers back to the source of the substrate. This process allowed confirmation of the observations recorded during the first segment of the foraging study and provided large samples of the foraged vegetation to be analyzed in the lab. After tracking the ants to the sources of their foraged material, samples of the living vegetation, or detritus, were collected and returned to the lab and stored in a freezer until analysis.

T. septentrionalis Nest Content Collection

To minimize the deleterious impact of nest collection on the *T. septentrionalis* populations, only one nest was gathered from each of the four stable colonized areas. Nest materials were collected by first aspirating all *T. septentrionalis* entering and leaving a nest for a five-minute period prior to excavation of the nests. Nest excavation began by digging trenches adjacent to the entrances of the nests to be sampled that were approximately 1m long, 1m deep, and 0.5 m wide. Following the completion of the trench, soil removal began across the apical surface of the nest. Soil was removed in

increments of 1-4 cm of depth, descending down the proximal wall of the trench, and a surface area of approximately 0.5 m^2. All attempts were made to maintain the entrance tunnel at a central location within the excavation site. Any ants encountered during this excavation process were immediately aspirated and collected. As chambers were encountered, care was taken to prevent the chambers from collapsing; any fungal material was removed and placed in storage and digging continued as new tunnels were exposed. Excavation at each nest continued until no further tunnels or chambers could be located.

Carbon/Nitrogen Composition

All analyses of C and N were performed by Peter Bernhardt in the Department of Ocean, Earth, and Atmospheric Sciences using a Europa 20-20 mass spectrometer equipped with an ANCA-GSL preparation module. The equipment was calibrated using ammonium sulfate (12.5 μg N) and sucrose (100 μg C). Each sample to be tested was placed an individual aluminum pellet and sealed. Preliminary samples were provided to assure adequate mass for analysis.

Macronutrient and Micronutrient Composition

Macronutrient, micronutrient, and heavy metal analyses for *T. septentrionalis*, forage leaf samples, nest fungus, and waste were performed by the Louisiana State University Agricultural Center. Sample. Sample masses of 0.5 g were mixed with 5.0 ml concentrated HNO_3 and 3.0 ml H_2O_2 and digested for 2.75 hr on heat block. Following digestion, samples were analyzed on an inductively coupled plasma atomic emission spectroscope (ICP-AES).

Caloric Composition

Calorimetry data were collected using a Parr 1281 calorimeter in the lab of Dr. Harold Rowe at Norfolk State University. All samples were pressed into pellets of approximately 0.5 g with a Parr pellet press (2811). Factory issued instructions were used to operate the equipment. Before samples were tested, a benzoic acid pellet was placed in the calorimeter bomb to standardize the calorie calculations. This test resulted in a standardization curve (Figure 4) for time (min) and temperature change (°C). Following standardization, each sample to be tested was weighed and placed in a stainless steel capsule where an igniting string was tied that connected the sample to the ignition electrodes. The electrode array was then screwed into the bomb jacket, and the ignition sequence was begun. Sample ID and weight were entered into the computer. The bombing apparatus was then filled with O_2 gas, and the sample was ignited. The resulting temperature rise of the bomb could be observed, but all calculations were performed by the calorimeter. Ignition and temperature measurement lasted eight minutes per sample. Due to limited sample masses, replicates were not possible for many of the samples.

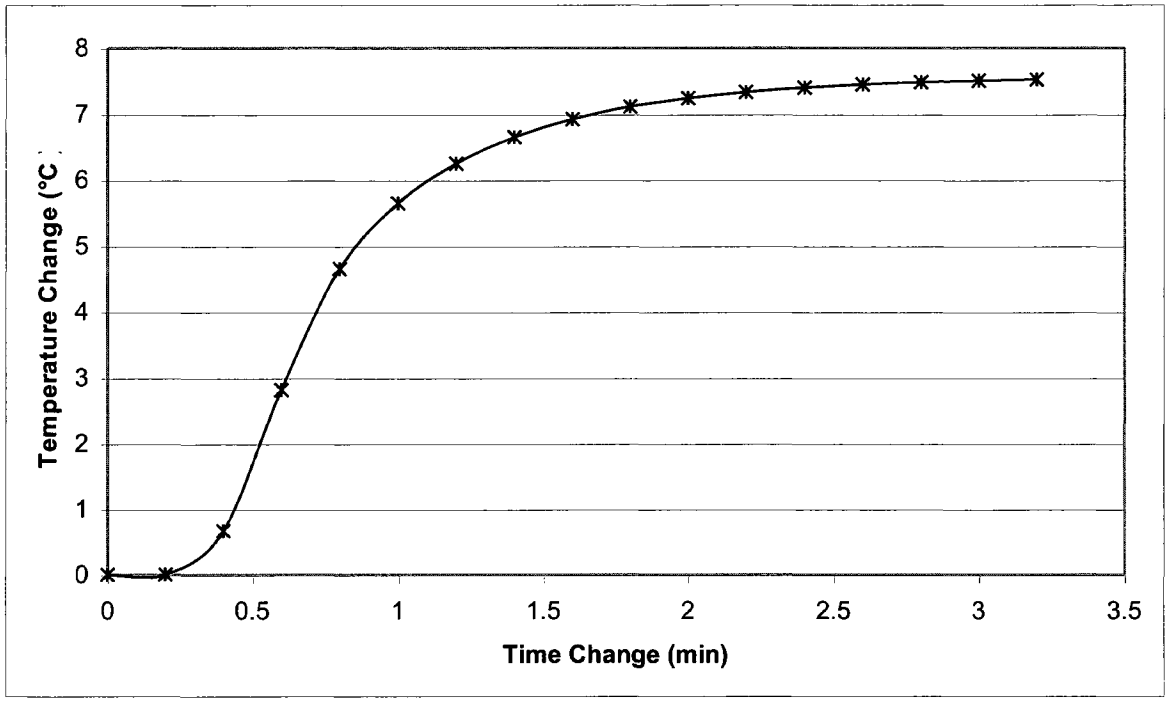

FIG. 4. Standard temperature curve for the analysis of calorimetric data. The X-axis identifies time change in increments of 0.2 minutes, and the Y-axis indicates changes in temperature (°C).

Statistical Analysis

All statistical analyses were performed using SPSS versions 10.0-15.0. Types of analyses performed included: ANOVA, logistic and linear regression analysis, and correlation analysis. Multifactoral analyses were performed primarily on abiotic conditions and seasonal biotic studies when comparisons between multiple dates, times of day, or fire regimes produced confounding variables. Community structure was compared using Shannon's Diversity Index and Shannon's Evenness Index.

CHAPTER III

RESULTS

Between 2001 and 2007 the study areas were subjected to nine controlled burns (Table 1). These burns may have affected multiple facets of this study; therefore, any segment of the study that was interrupted by burning has been noted, and those pre- and post-burn data analyzed separately.

ABIOTIC CONDITIONS – ABOVE GROUND

Temperature

Soil (B=0.0136; S.E.=0.027; Wald=24.626; df=1; $p<0.001$; Exp(B)=1.145) and air temperatures (B=0.0139; S.E.=0.026; Wald=27.761; df=1; $p<0.001$; Exp(B)=1.150) exhibited a significant influence on the activity patterns of *Trachymyrmex septentrionalis* (Table 2). Over the two-year collection period, soil temperatures ranged from 13.2 to 37.0°C, with a mean of 23.5°C. Ant activity was continuous throughout a range of 13.4 – 33.5°C, with the mean soil temperature for activity at 22.3°C (Figure 5). The results for air temperature were similar to soil temperature. The overall range was 13.4 - 38.6°C, with a mean of 24.6°C, but ant activity was limited to a range of 13.4 to 36.9°C, with a mean of 23.3°C (Figure 6); however, favorable temperatures did not ensure that ants would be active. Ants were inactive at mean temperatures of 25.1 and 26.4°C for soil and air, respectively.

TABLE 2. Effect of abiotic conditions on activity of *T. septentrionalis*. Mean values collected from active and inactive nests over a two-year period. Values in parenthesis (*) indicate the number of data points used to calculate each value.

Ts Activity	Mean Soil Temperature (°C)	Mean Air Temperature (°C)	Mean Light Intensity (lumen)	Mean Air Humidity (%)
Yes	22.3 (175)	23.3 (175)	302.8 (81)	69.3 (52)
No	25.1 (130)	26.4 (130)	595.2 (45)	65.6 (41)

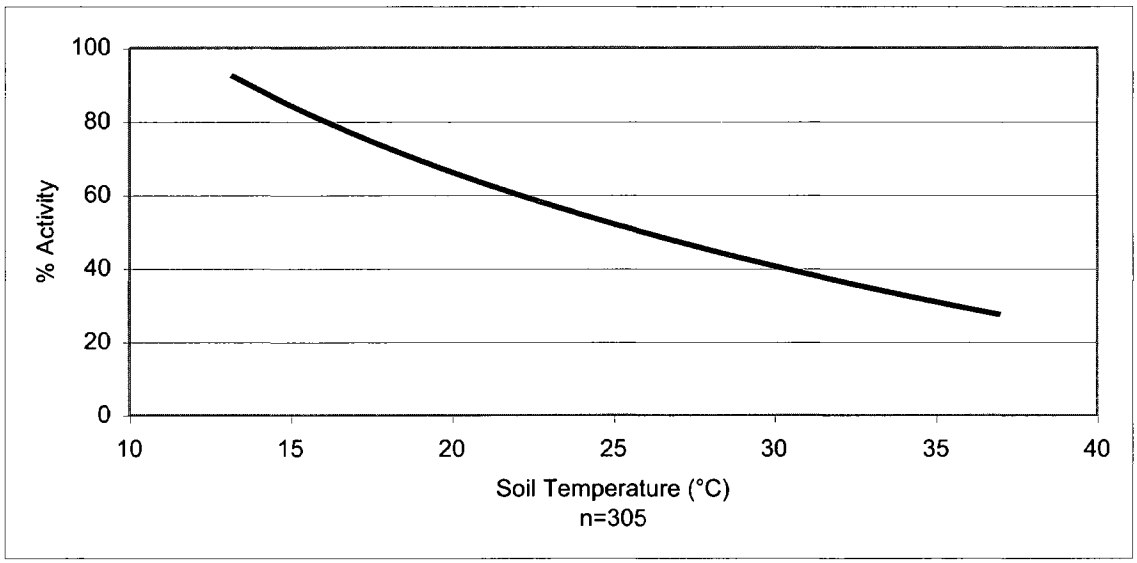

FIG. 5. Effect of soil temperature on *T. septentrionalis* activity during the two-year study.

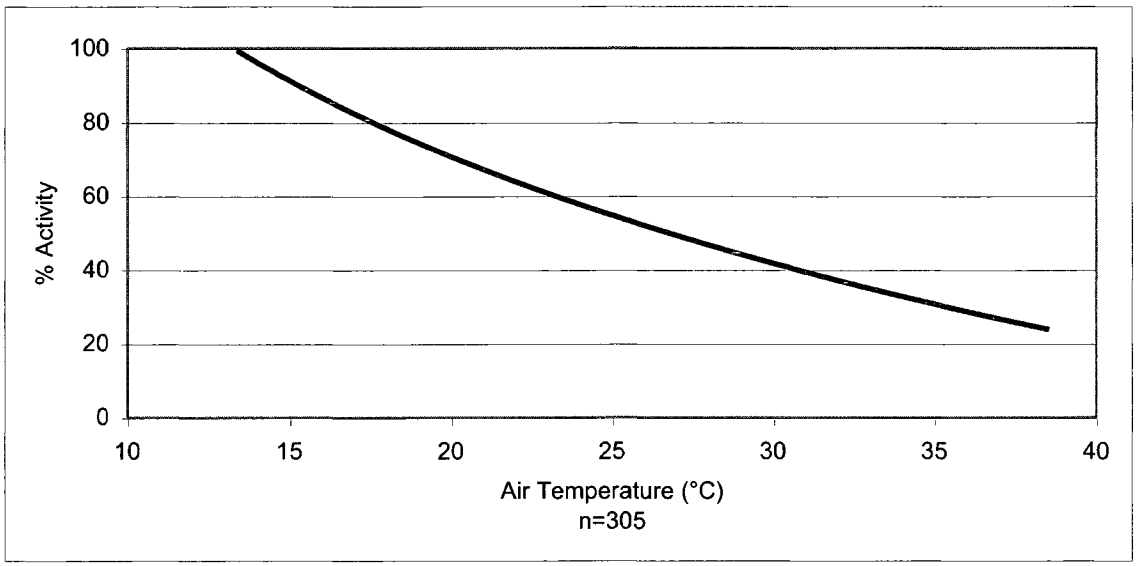

FIG. 6. Effect of air temperature on *T. septentrionalis* activity during the two-year study.

Nest activity was compared by analyzing soil and air temperature data of individual months before and after controlled burns. A binomial logistic regression analysis of data (month x activity) from individual months before the controlled burn of May 31, 2001 (Table 3) found that only May 2001 produced significantly lower active temperatures than inactive temperatures: 18.7°C versus 26.1°C for soil (B=0.668; S.E.=0.190; Wald=12.354; df=1; $p<0.001$; Exp(B)=1.950) and 20.8°C versus 27.6°C for air (B=0.356; S.E.=0.093; Wald=14.618; df=1; $p<0.001$; Exp(B)=1.427) (Figures 7 and 8).

TABLE 3. Effect of abiotic conditions on activity of *T. septentrionalis* before the controlled burn of May 31, 2001. Mean values collected from active and inactive nests over a one and a half year period. Values in parenthesis (*) indicate the number of data points used to calculate each value.

Active	Year	Month	Mean Soil Temperature (°C)	Mean Air Temperature (°C)	Mean Light Intensity (lumen)
Yes	2000	July	28.9 (5)	28.9 (5)	N/A
		August	25.7 (10)	26.4 (10)	N/A
		September	23.4 (10)	22.8 (10)	N/A
		October	27.1 (5)	28.1 (5)	N/A
		November	21.4 (1)	22.3 (1)	N/A
	2001	April	26.1 (3)	27.0 (3)	377.8 (3)
		May	18.7 (43)	20.8 (43)	156.9 (22)
	Total		21.8 (77)	23.1 (77)	183.4 (25)
No	2000	July	28.9 (13)	30.1 (13)	N/A
		August	28.7 (12)	29.6 (12)	N/A
		September	24.1 (11)	23.3 (11)	N/A
		October	27.5 (19)	28.4 (19)	N/A
		November	N/A	N/A	N/A
	2001	April	25.1 (4)	26.9 (4)	628.3 (4)
		May	26.1 (21)	27.6 (21)	676.3 (17)
	Total		26.9 (80)	27.9 (80)	667.1 (21)

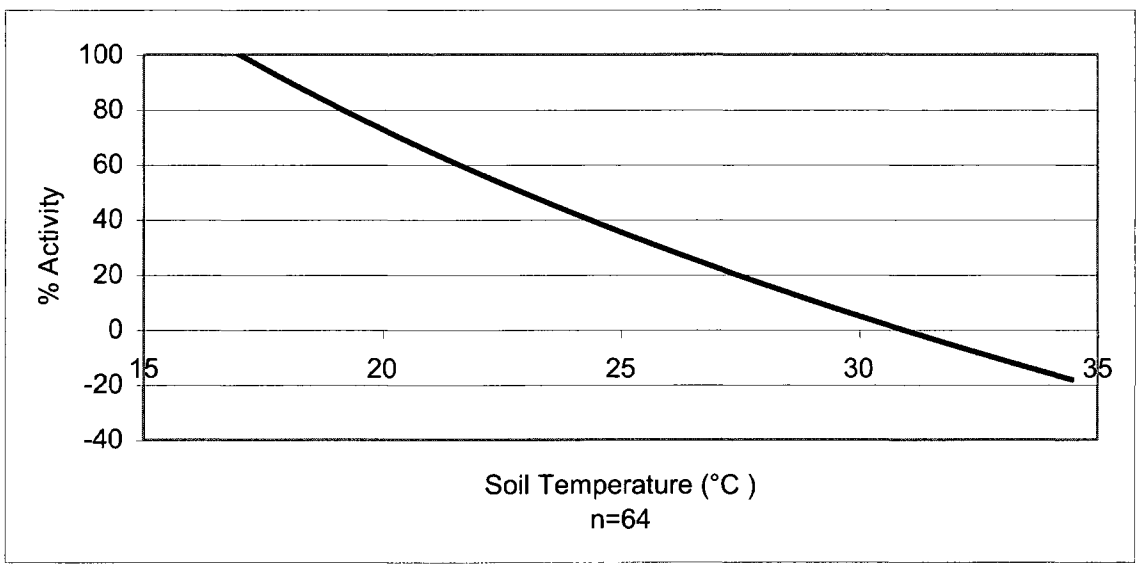

FIG. 7. Effect of soil temperature on *T. septentrionalis* activity during May 2001 before the controlled burn of May 31.

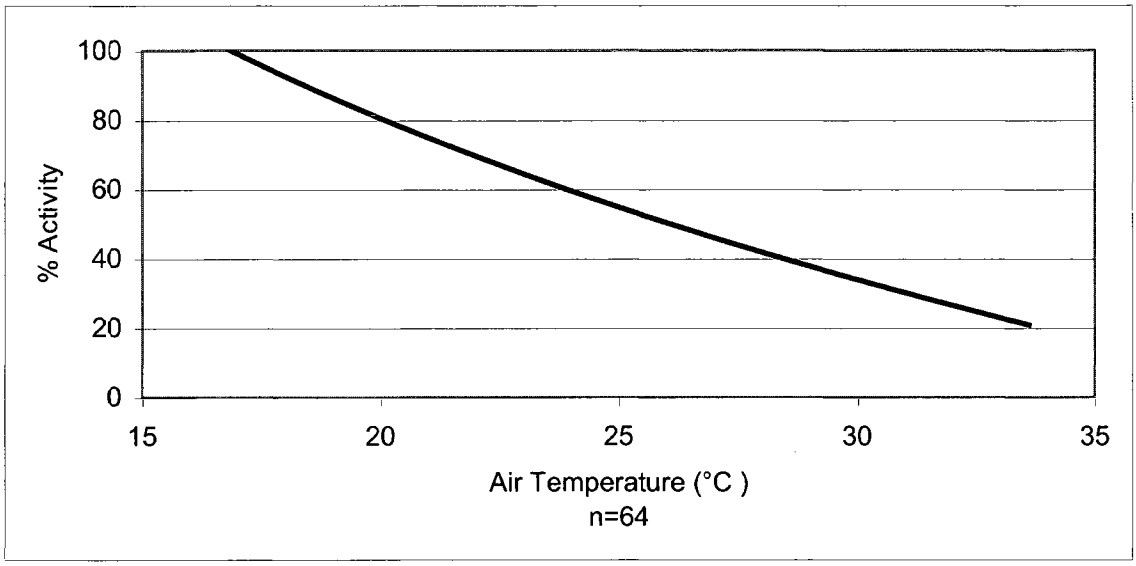

FIG. 8. Effect of air temperature on *T. septentrionalis* activity during May 2001 before the controlled burn of May 31.

Temperature data, as well as other portions of the abiotic study, were also divided in their analysis because controlled burning took place across a section of the study area at the end of the May 2001 collection period (Table 4). The entire preserve was not subject to the controlled burn, so comparisons could be made between burned and unburned areas. Binomial logistic regression analysis did not indicate a significant effect of fire on soil temperature (B=-0.011; S.E.=0.074; Wald=0.021; df=1; p=0.884; Exp(B)=0.989), air temperature (B=0.040; S.E.=0.054; Wald=0.532; df=1; p=0.466; Exp(B)=1.040), light intensity (B=0.001; S.E.=0.002; Wald=0.144; df=1; p=0.704; Exp(B)=1.001), or on activity patterns (B=0.510; S.E.=0.437; Wald=1.365; df=1; p=0.243; Exp(B)=1.666) of *T. septentrionalis*, but fire did significantly influence air humidity (B=-0.079; S.E.=0.027; Wald=8.602; df=1; p=0.003; Exp(B)=0.924).

TABLE 4. Effect of abiotic conditions on activity of *T. septentrionalis* immediately after the controlled burn of May 31, 2001. Mean values collected from active and inactive nests over a five-week period following a controlled burn. Values in parenthesis (*) indicate the number of data points used to calculate each value.

Active	Month	Fire	Mean Soil Temperature (°C)	Mean Air Temperature (°C)	Mean Light Intensity (lumen)	Mean Air Humidity (%)
Yes	June	Yes	20.7 (10)	20.0 (10)	136.8 (10)	N/A
		No	23.5 (36)	23.3 (36)	175.3 (22)	N/A
	August	Yes	26.8 (13)	28.4 (13)	396.3 (3)	59.6 (13)
		No	26.0 (17)	28.4 (17)	248.0 (5)	70.5 (17)
No	June	Yes	20.6 (2)	20.4 (2)	130.0 (2)	N/A
		No	23.0 (7)	22.9 (7)	145.6 (5)	N/A
	August	Yes	26.1 (11)	29.2 (11)	316.0 (2)	52.7 (11)
		No	26.9 (12)	28.4 (12)	244.0 (2)	66.0 (12)

Date of data collection was shown to significantly affect soil and air temperature (B=-1.422; S.E.=0.492; Wald=8.354; df=1; p=0.004; Exp(B)=0.241 and B=-0.376; S.E.=0.186; Wald=4.071; df=1; p=0.044; Exp(B)=0.687, respectively) during the fourth and fifth month following the burn of May 31, 2001. Activity patterns were significantly influenced by changes in soil (B=0.015; S.E.=0.003; Wald=26.084; df=1; p<0.001; Exp(B)=1.015) and air (B=0.015; S.E.=0.003; Wald=28.451; df=1; p<0.001; Exp(B)=1.015) temperature between months of data collection (Table 5).

TABLE 5. Effect of abiotic conditions on activity of *T. septentrionalis* three months after the controlled burn of May 31, 2001. Mean values collected from active and inactive nests during the final two-month collection period of 2001. Values in parenthesis (*) indicate the number of data points used to calculate each value.

Active	Month	Mean Soil Temperature (°C)	Mean Air Temperature (°C)	Mean Light Intensity (lumen)	Mean Air Humidity (%)
Yes	September	18.7 (12)	19.2 (12)	76.7 (6)	73.8 (12)
	October	15.2 (10)	17.3 (10)	1182.5 (10)	74.6 (10)
	Total	17.1 (22)	18.3 (22)	767.8 (16)	74.1 (22)
No	September	18.0 (10)	19.0 (10)	76.0 (5)	76.2 (10)
	October	14.5 (8)	18.3 (8)	1285.6 (8)	69.4 (8)
	Total	16.8 (18)	18.7 (18)	820.3 (13)	73.2 (18)

When data from 2000 and 2001 were combined for October (Tables 3 and 5), soil temperatures for active nests (19.2°C) were significantly lower (B=0.104; S.E.=0.052; Wald=3.971; df=1; p=0.046; Exp(B)=1.110) than inactive nests (23.6°C). Air temperatures for active nests (20.9°C) were also significantly lower (B=0.144; S.E.=0.063; Wald=5.183; df=1; p=0.023; Exp(B)=1.155) than inactive nests (25.4°C) (Figures 9 and 10).

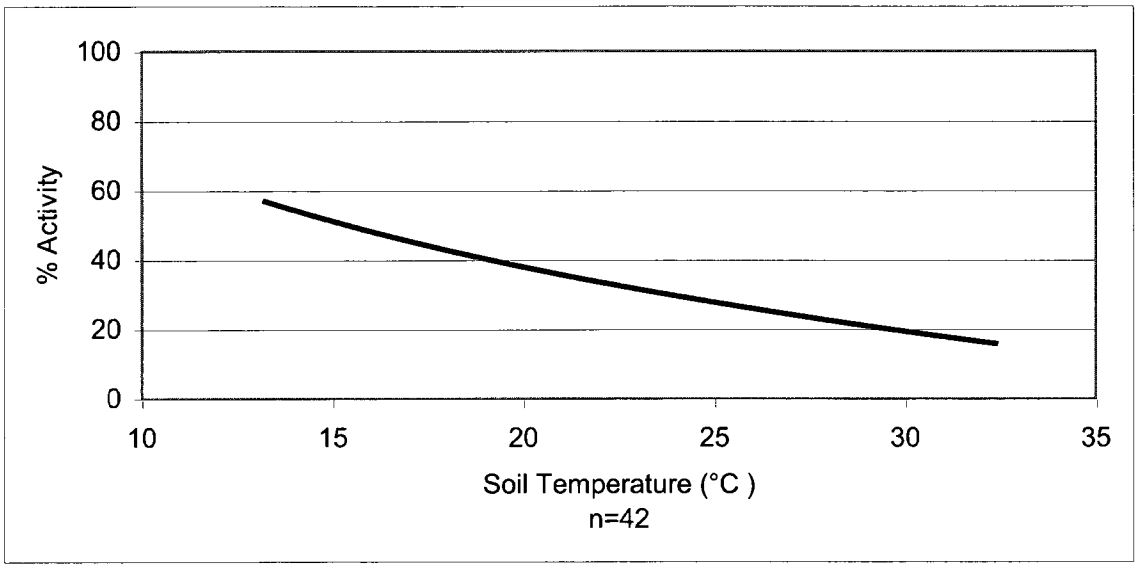

FIG. 9. Effect of soil temperature on *T. septentrionalis* activity during October 2000, before the controlled burn of May, 2001, and October 2001, four months after the controlled burn.

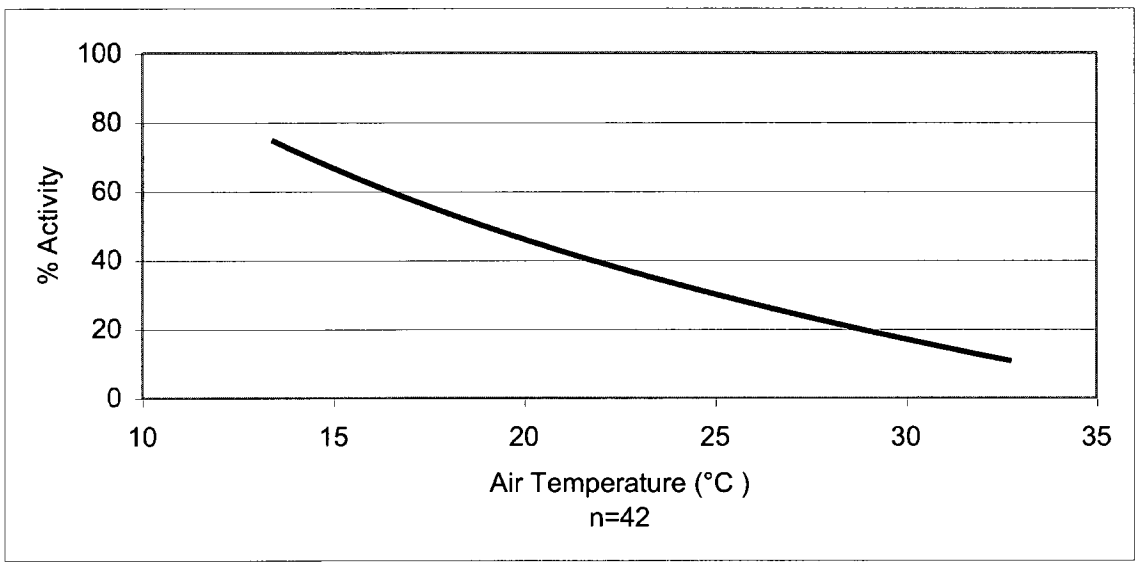

FIG. 10. Effect of air temperature on *T. septentrionalis* activity during October 2000, before the controlled burn of May, 2001, and October 2001, four months after the controlled burn.

Due to changes in scheduling during the study, data collection times frequently changed between AM and PM. Both soil (B=-0.324; S.E.=0.042; Wald=60.575; df=1; p<0.001; Exp(B)=1.155) and air temperatures (B=-0.207; S.E.=0.032; Wald=42.708; df=1; p<0.001; Exp(B)=0.813) changed significantly in relation to the time of the measurement (Table 6). Mean PM temperatures produced consistently higher values than AM temperatures for both soil, 27.0°C (n=14) versus 21.6°C (n=57), and air, 27.5°C (n=14) versus 23.2°C (n=57). However, only PM mean soil temperatures were associated with a significant impact (B=0.156; S.E.=0.061; Wald=6.568; df=1; p=0.010; Exp(B)=1.168) on activity; 25.6°C for active nests and 27.7°C for inactive nests (Figure 11).

TABLE 6. Effect of time of day on data collection on abiotic conditions and their subsequent effect on activity of *T. septentrionalis* before controlled burn. Mean values collected from active and inactive nests over a two-month period beginning the 2001 collection season. Values in parenthesis (*) indicate the number of data points used to calculate each value.

Active	Month	Time	Mean Soil Temperature (°C)	Mean Air Temperature (°C)	Mean Light Intensity (lumen)
Yes	April	AM	N/A	N/A	N/A
		PM	26.1 (3)	27.0 (3)	377.8 (3)
	May	AM	18.7 (43)	20.8 (43)	156.9 (22)
		PM	N/A	N/A	N/A
No	April	AM	N/A	N/A	N/A
		PM	25.1 (4)	26.9 (4)	628.3 (4)
	May	AM	23.3 (14)	26.1 (14)	387.0 (6)
		PM	31.6 (7)	30.6 (7)	834.1 (11)
	Total	AM	23.3 (14)	26.1 (14)	387.0 (6)
		PM	29.2 (11)	29.3 (11)	779.2 (15)

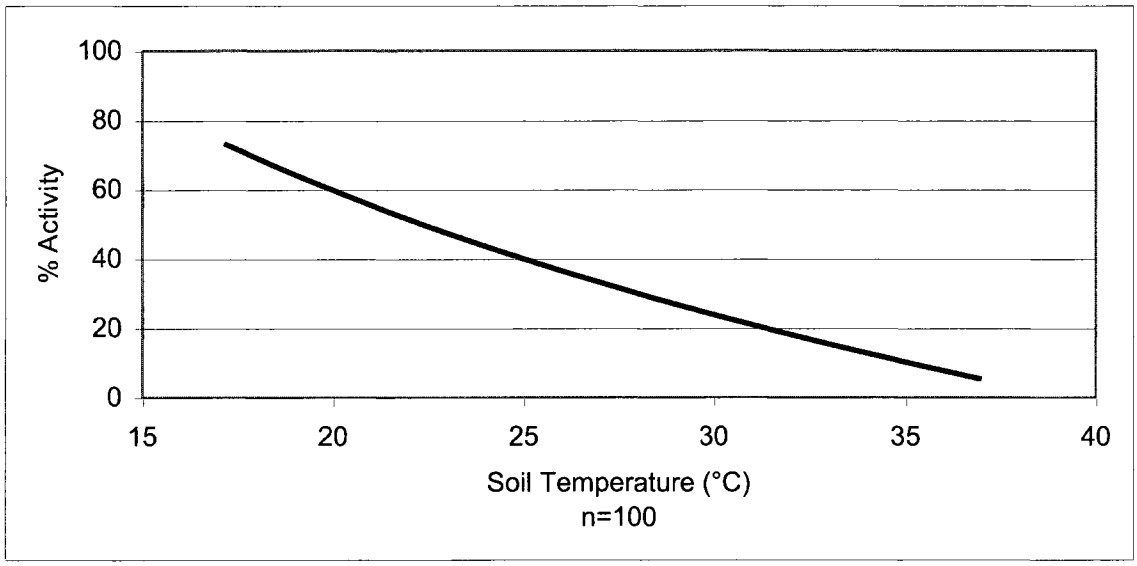

FIG. 11. Effect of PM soil temperature on *T. septentrionalis* activity 2000 and 2001, before the controlled burn of May 31, 2001.

Air Humidity

Activity by *T. septentrionalis* was not significantly affected by air humidity (Table 2). The ranges for percent humidity differed by only a single percentage point between active nests and inactive nests, 43% - 93% (mean=69.3%) and 42% - 93% (mean=65.6%), respectively (Figure 12). No significant relationships were found between the controlled burn (Table 4), or time of day (Table 6), and *T. septentrionalis* nest activity. Only October 2001 (Table 5) produced a significant difference (B=-0.347; S.E.=0.173; Wald=4.040; df=1; p=0.044; Exp(B)=0.707) between the mean air humidity of 74.6% at active nests and 69.4 at inactive nests (Figure 13)

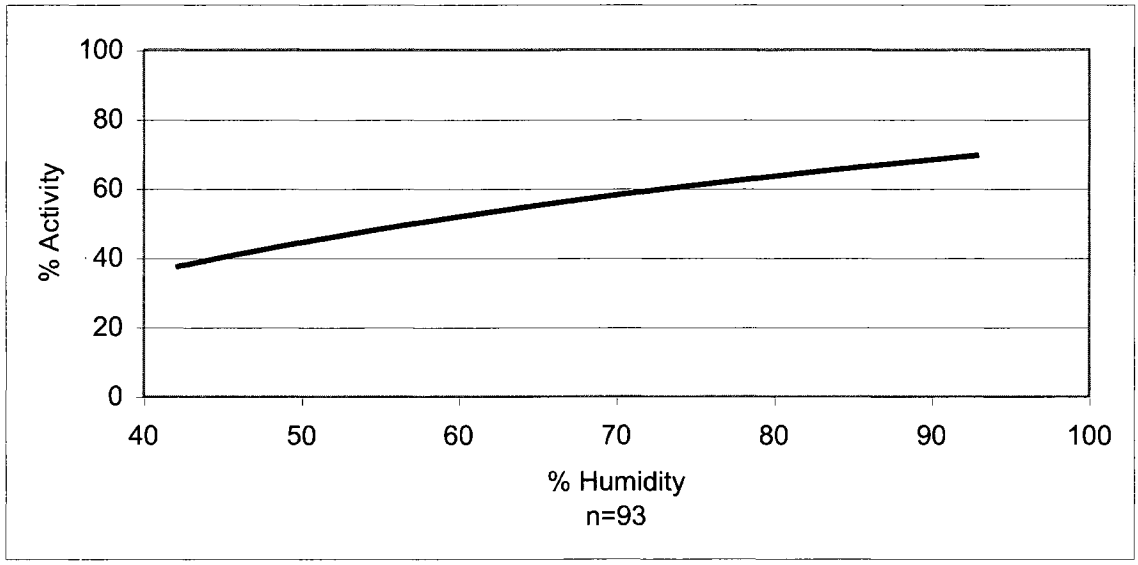

FIG. 12. Effect of air humidity on *T. septentrionalis* activity during the two-year study.

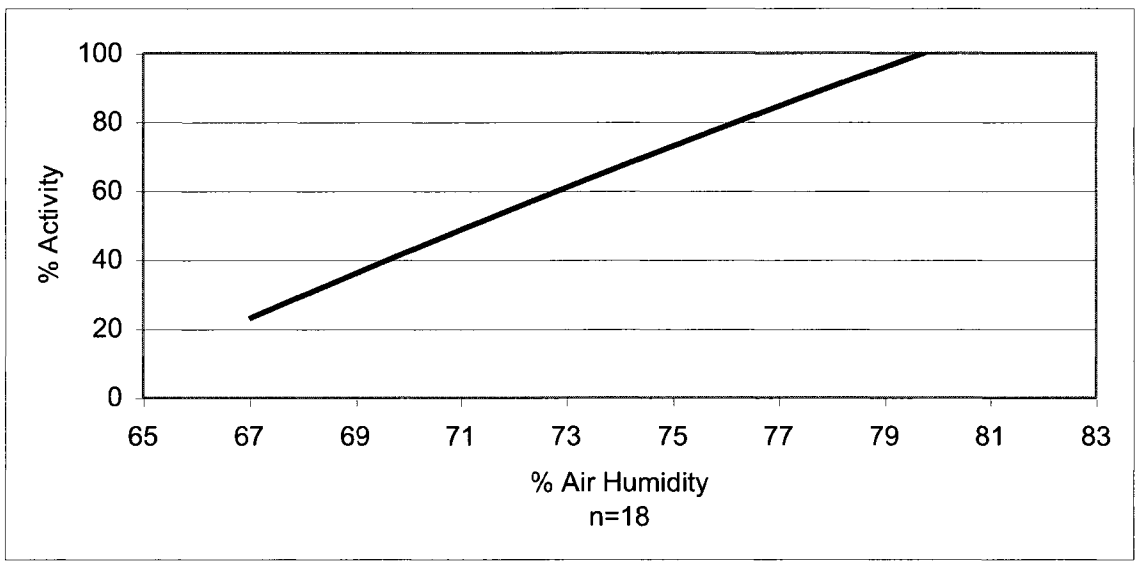

FIG. 13. Effect of air humidity on *T. septentrionalis* activity during October 2001.

Light Intensity

Light intensity did have a significant influence (B=0.001; S.E.<0.001; Wald=9.186; df=1; p=0.002; Exp(B)=1.001) on activity patterns (Table 2). *T. septentrionalis* nests were active at light intensity levels ranging from 2651.0 lumens to 19.8 lumens with a mean of 302.8 lumens, but data from all inactive nests were also within this range (2057.0 lumens - 33.0 lumens), producing a mean of 595.2 lumens (Figure 14).

Light intensity was also analyzed concerning the same factors as temperature: occurrence of a controlled burn, time of day, and individual month. Only data collected before the controlled burn (Table 3) resulted in a significant difference (B=0.006; S.E.=0.002; Wald=15.176; df=1; p<0.001; Exp(B)=1.006) in light intensity for active nests (183.4 lumens) and for inactive nests (667.1 lumens) (Figure 15). While there was a significant difference (B=-0.001; S.E.<0.001; Wald=7.104; df=1; p=0.008; Exp(B)=0.999) between AM and PM light intensity levels (Table 6), these differences were not significant in reference to activity patterns. During May 2001 (Table 3), the mean light intensity level for active nests was 156.9 lumens while the mean over inactive nests was 676.3 lumens, which produced a significant difference (B=0.007; S.E.=0.002; Wald=12.252; df=1; p<0.001; Exp(B)=1.007) (Figure 16).

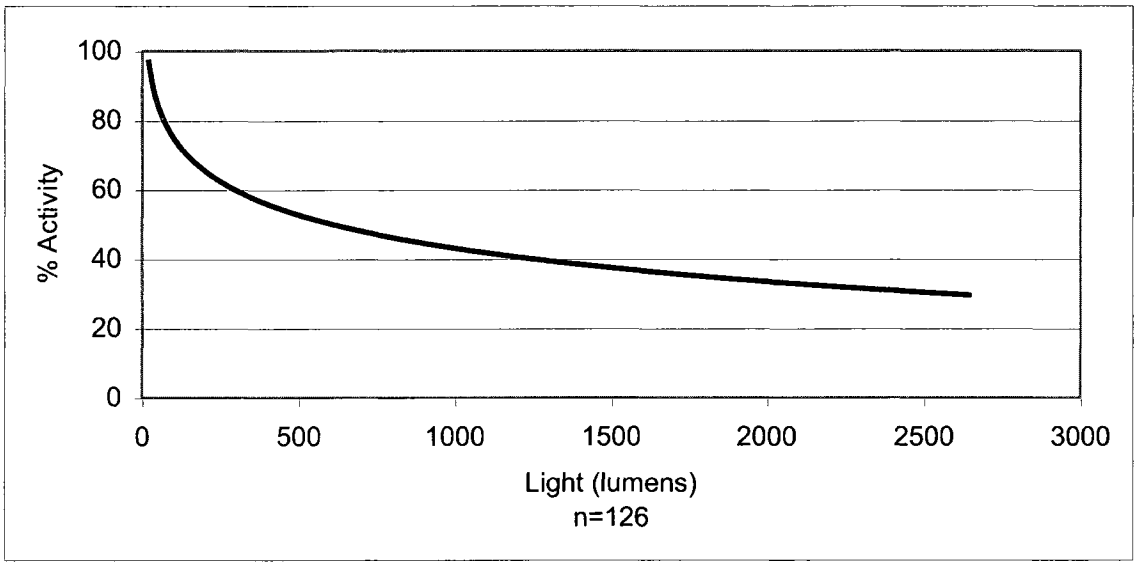

FIG. 14. Effect of light intensity on *T. septentrionalis* activity during the two-year study.

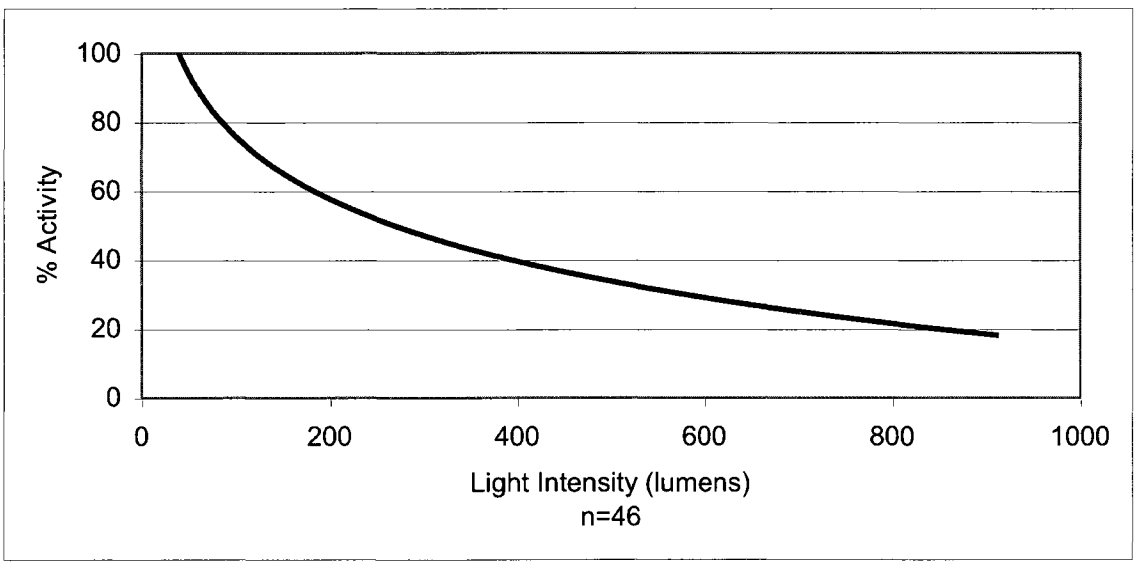

FIG. 15. Effect of light intensity on *T. septentrionalis* activity before the controlled burn of May 31, 2001

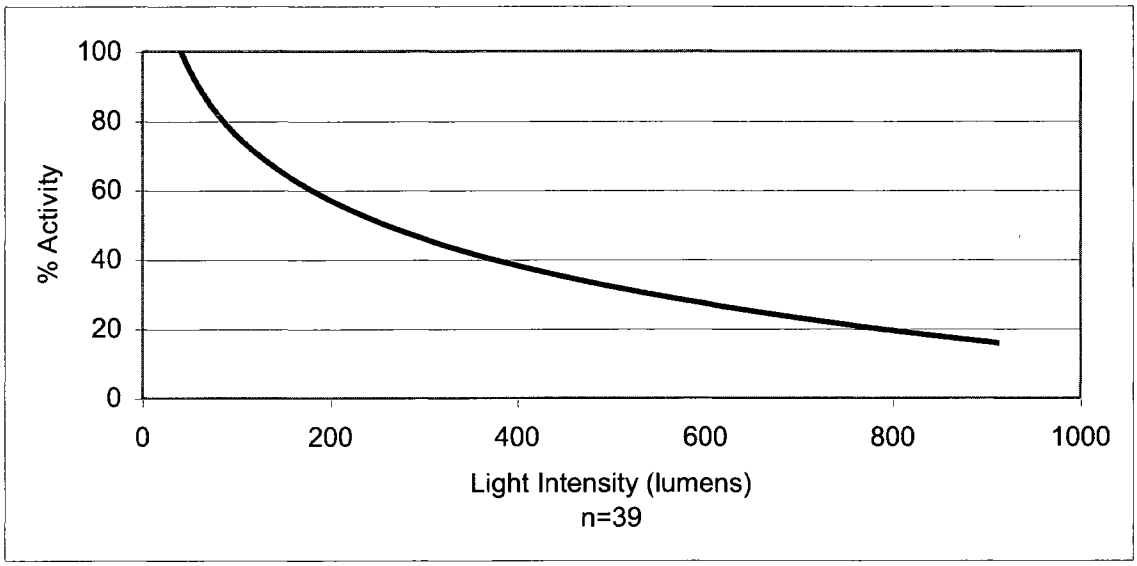

FIG. 16. Effect of light intensity on *T. septentrionalis* activity during May 2001

Vegetation Cover

Vegetation cover (Table 7) at the ground level was significantly greater (B=-0.033; S.E.=0.013; Wald=6.161; df=1; p=0.013; Exp(B)=0.967) above active *T. septentrionalis* nests (71.1%) than above inactive nests (57.9%) before the controlled burn in July 2001 (Figure 17). During this time period, ground cover was negatively correlated with soil temperature (r=-0.359; p=0.003), air temperature (r=-0.304; p=0.014), and light intensity (r=-0.395; p=0.012), but positively correlated with ground cover (r=0.427; p=0.002). Following the controlled burn, there was no significant difference (B=-0.013; S.E.=0.016; Wald=0.603; df=1; p=0.437; Exp(B)=0.988) between active (63.9%) and inactive nests (59.0%) (Figure 18) in vegetative cover. Chest level vegetation was not significantly different between active and inactive nests before (B=-0.010; S.E.=0.010; Wald=0.872; df=1; p=0.350; Exp(B)=0.990), or after (B=0.002; S.E.=0.019; Wald=0.013; df=1; p=0.909; Exp(B)=1.002), the controlled burn.

TABLE 7. Effect of vegetation cover, at the ground and chest level, on *T. septentrionalis* activity before and after a controlled burn in May 2001. Bars represent standard error. Numbers in parenthesis (*) indicate sample sizes.

Active	Treatment	Mean Percent Ground Cover (%)	Mean Percent Chest Cover (%)
Yes	Preburn	71.1 (42)	58.0 (45)
	Postburn	63.9 (22)	65.7 (22)
No	Preburn	57.9 (27)	52.4 (26)
	Postburn	59.0 (18)	66.3 (17)

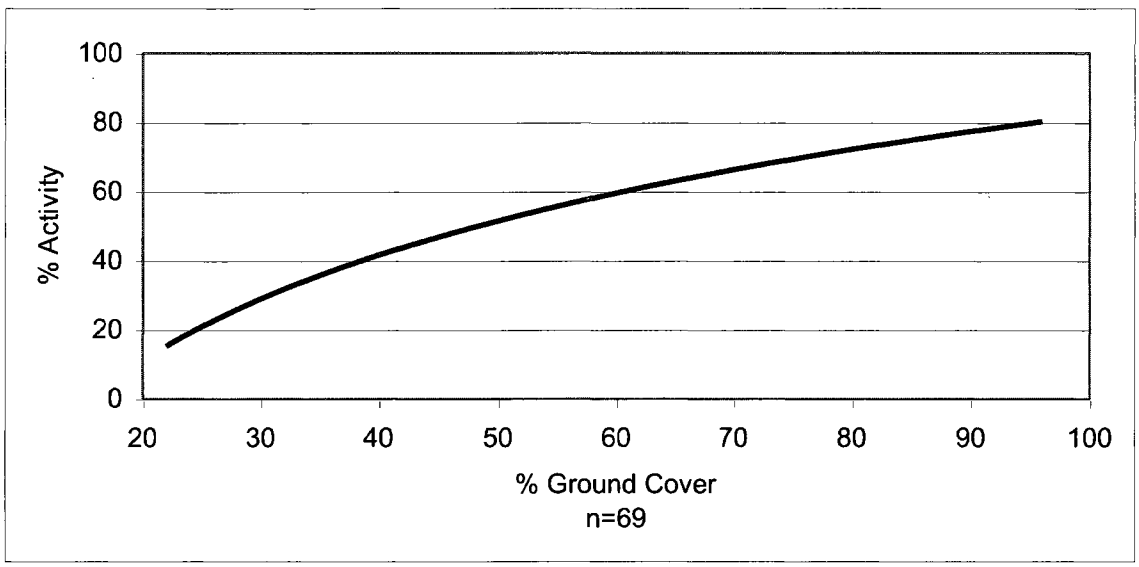

FIG. 17. Effect of ground cover on *T. septentrionalis* activity prior to a controlled burn in 2001.

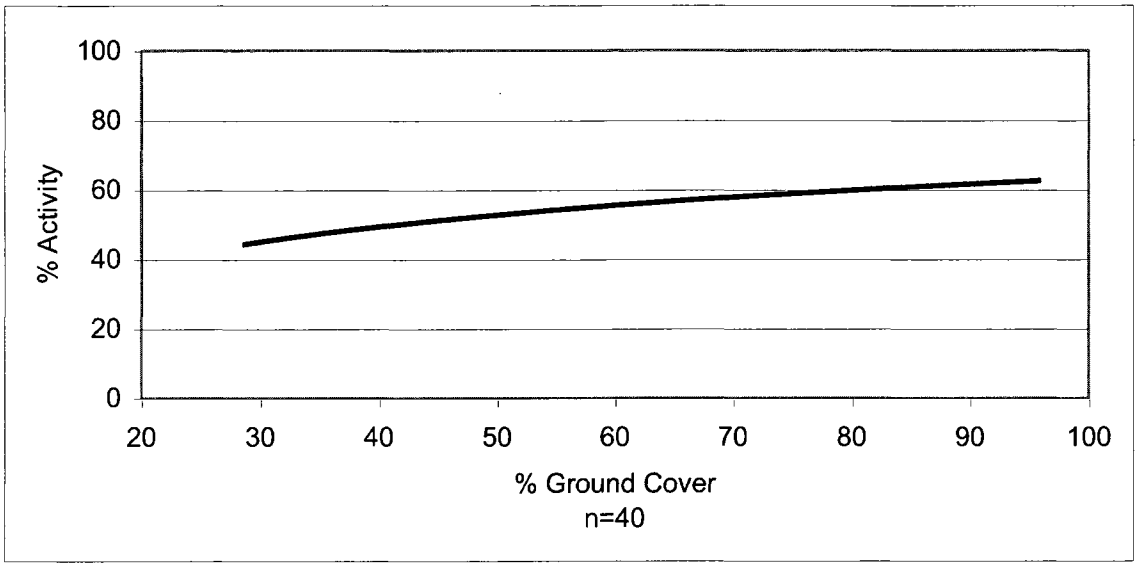

FIG. 18. Effect of ground cover on *T. septentrionalis* activity after a controlled burn in 2001.

ABIOTIC CONDITIONS – BELOW GROUND

Water Content

Data collected over two years for soil moisture were subject to a change in equipment between 2003 and 2004. In year one of the study, soil moisture was not significantly different between treatments of areas with *T. septentrionalis* and areas without *T. septentrionalis* (B=0.063; S.E.=0.033; Wald=3.541; df=1; p=0.060; Exp(B)=1.065), but significantly higher moistures were found in successive soil depths: top-middle (B=0.368; S.E.=0.087; Wald=18.056; df=1; p<0.001; Exp(B)=1.445) and middle-bottom (B=0.444; S.E.=0.132; Wald=11.419; df=1; p=0.001; Exp(B)=1.560). When both variables were combined in a multifactor analysis, the water content values were significantly lower in areas with *T. septentrionalis* than in those without *T. septentrionalis* for only the top layer of soil (B=0.437; S.E.=0.167; Wald=6.841; df=1; p=0.009; Exp(B)=1.548) (Table 8). Figure 19 depicts the relationship between water

content at different soil levels and the presence of *T. septentrionalis* within BEP during 2003.

TABLE 8. Effect of abiotic conditions below the soil surface on presence or absence of *T. septentrionalis* colonies. Mean values collected from populated and unpopulated areas over a two-year period. Values in parenthesis (*) indicate the number of data points used to calculate each value.

Treatment	Year	Soil Level	Mean % Organic Matter	Mean pH	Mean % Soil Water	Mean CEC (meq/ 100g)	Mean Soil Composition (%)		
							Sand	Silt and Clay	Misc.
Populated	2003	Top	1.4 (15)	6.339 (15)	6.2 (15)	130.6 (3)	99.5 (3)	0.07874 (3)	0.4005 (3)
		Middle	0.4 (15)	6.319 (15)	16.5 (15)	163.9 (3)	99.7 (3)	0.2746 (3)	0.02453 (3)
		Bottom	0.7 (15)	6.277 (15)	22.1 (15)	202.7 (3)	99.7 (3)	0.1902 (3)	0.07756 (3)
	2004	Top	N/A	6.813 (30)	N/A	N/A	N/A	N/A	N/A
		Middle	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Bottom	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Unpopulated	2003	Top	1.2 (15)	6.332 (15)	11.6 (15)	163.9 (3)	99.5 (3)	0.2111 (3)	0.3429 (3)
		Middle	0.4 (15)	6.337 (15)	19.3 (15)	194.4 (3)	99.7 (3)	0.2140 (3)	0.07809 (3)
		Bottom	0.6 (15)	6.356 (15)	21.8 (15)	183.3 (3)	99.6 (3)	0.3662 (3)	0.03910 (3)
	2004	Top	N/A	6.607 (30)	N/A	N/A	N/A	N/A	N/A
		Middle	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Bottom	N/A	N/A	N/A	N/A	N/A	N/A	N/A

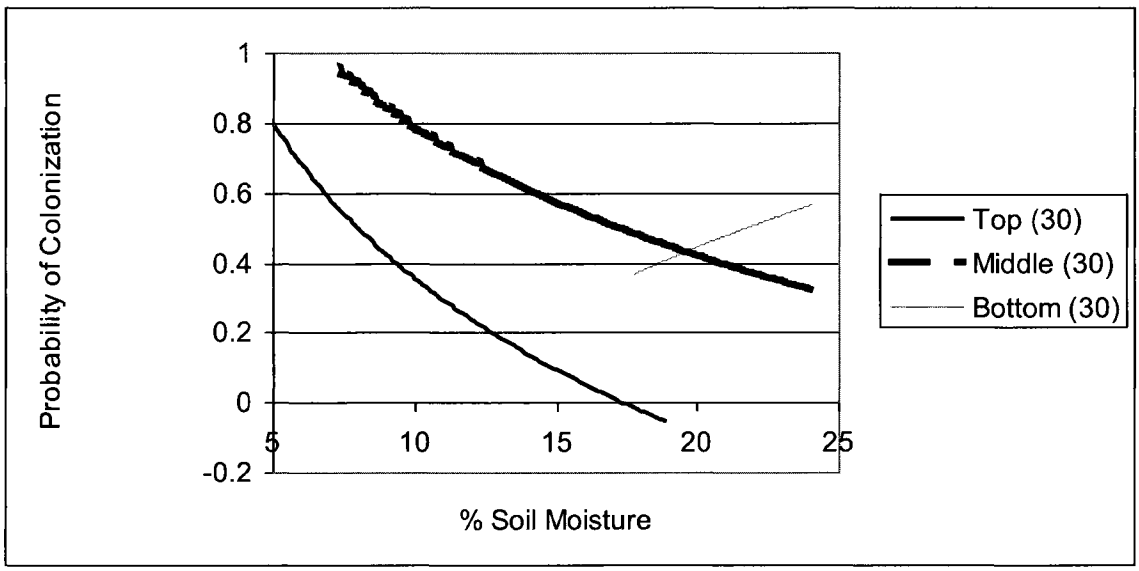

FIG. 19. Effect of water content in three layers of soil: Top (0-30 cm), Middle (30-60 cm), and Bottom (60-90 cm) on the presence or absence of *T. septentrionalis* colonies in 2003. Numbers in parenthesis (*) indicate sample sizes.

Due to a change in equipment between years one and two, which produced categorical data instead of numerical data, year two data were analyzed using ordinal regression analyses. Figure 20 shows that analyses of soil water content in the top layer of year two yielded significant differences between soils in populated and unpopulated areas (B=4.558; S.E.=0.953; Wald=22.861; df=1; $p<0.001$; Exp(B)=95.423). Significant differences were also found when treatments from 2003 (B=2.668; S.E.=0.524; Wald=25.910; df=1; $p<0.001$; Exp(B)=.14.405) and a combination of 2003-2004 (B=1.400; S.E.=0.564; Wald=6.170; df=1; p=0.013; Exp(B)=4.056) data were examined.

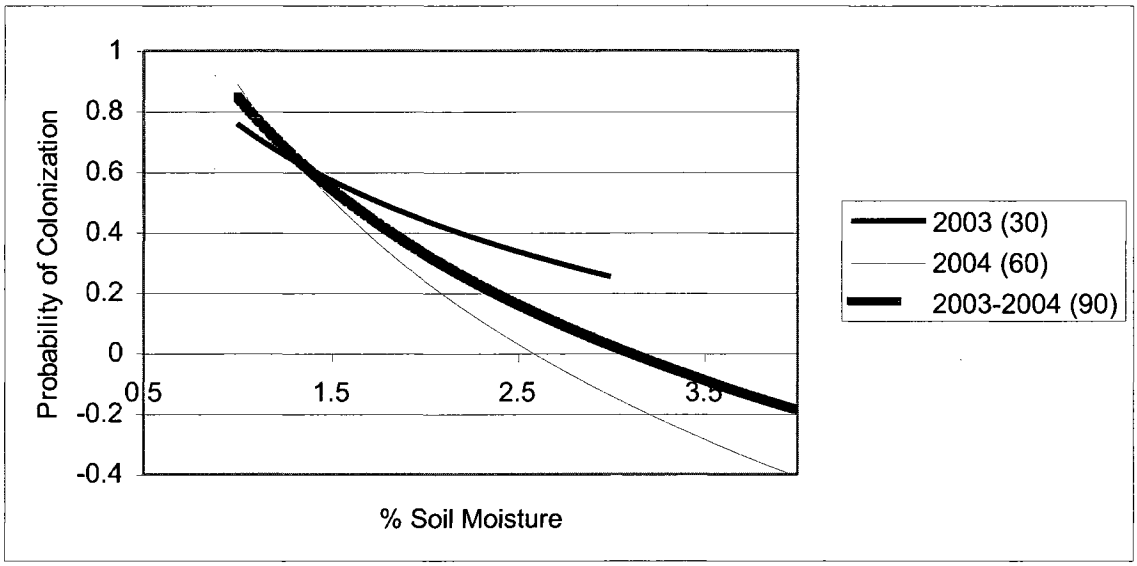

FIG. 20. Effect of ranked water content in the Top (0-30 cm) soil layer of different study seasons on the presence or absence of *T. septentrionalis* colonies in 2003 and 2004 using ranked values. Numbers in parenthesis (*) indicate sample sizes.

Reexamination of water content data from year one using a ranking analysis still produced no significant difference between populated and unpopulated areas without consideration of layers (Figure 21). Values from a combination of data from both years did produce significant differences (B=0.901; S.E.=0.214; Wald=17.728; df=1; $p<0.001$; Exp(B)=2.463) between areas with *T. septentrionalis* and those without.

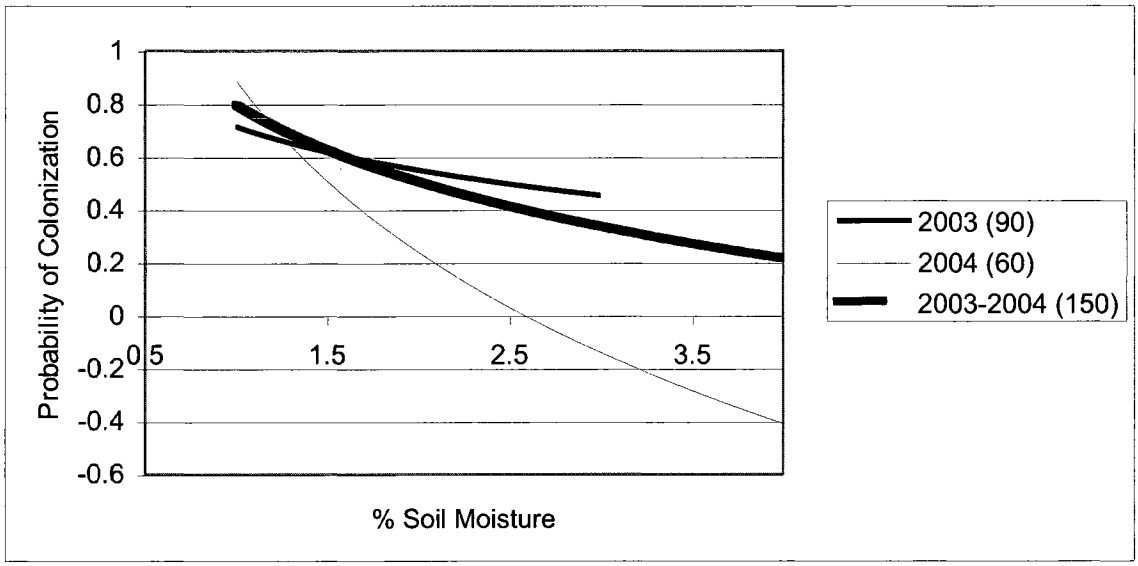

FIG. 21. Effect of ranked water content in all soil layers of different study seasons on the presence or absence of *T. septentrionalis* colonies in 2003 and 2004 using ranked values. Bars represent standard error. Numbers in parenthesis (*) indicate sample sizes.

Soil Texture

Soil composition was measured only during 2003 (Table 8). Sand (Figure 22), silt and clay (Figure 23), and miscellaneous (Figure 24) soil components were not found to be significantly different (sand: B=-1.329; S.E.=2.109; Wald=0.397; df=1; p=0.529; Exp(B)=.265; silt and clay: B=2.487; S.E.=2.787; Wald=0.796; df=1; p=0.372; Exp(B)=12.020; miscellaneous: B=-0.454; S.E.=2.674; Wald=0.029; df=1; p=0.865; Exp(B)=.635) between areas populated by *T. septentrionalis* and areas without *T. septentrionalis* colonies. No significant differences were found between soil components at different levels or between levels and the presence or absence of *T. septentrionalis* populations.

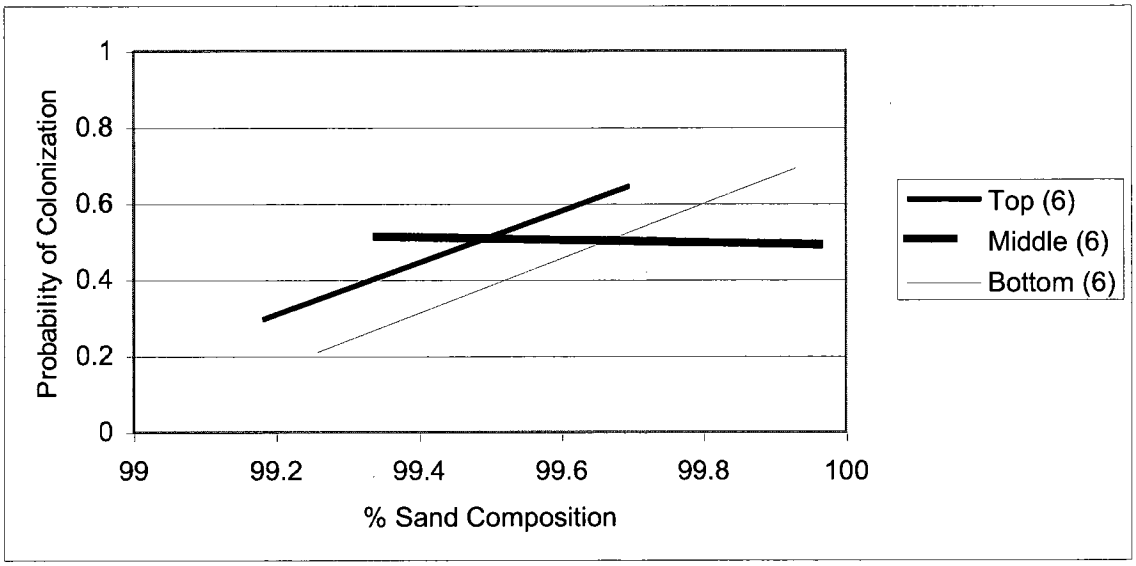

FIG. 22. Effect of sand in three layers of soil: Top (0-30 cm), Middle (30-60 cm), and Bottom (60-90 cm) on the presence or absence of *T. septentrionalis* colonies in 2003. Numbers in parenthesis (*) indicate sample sizes.

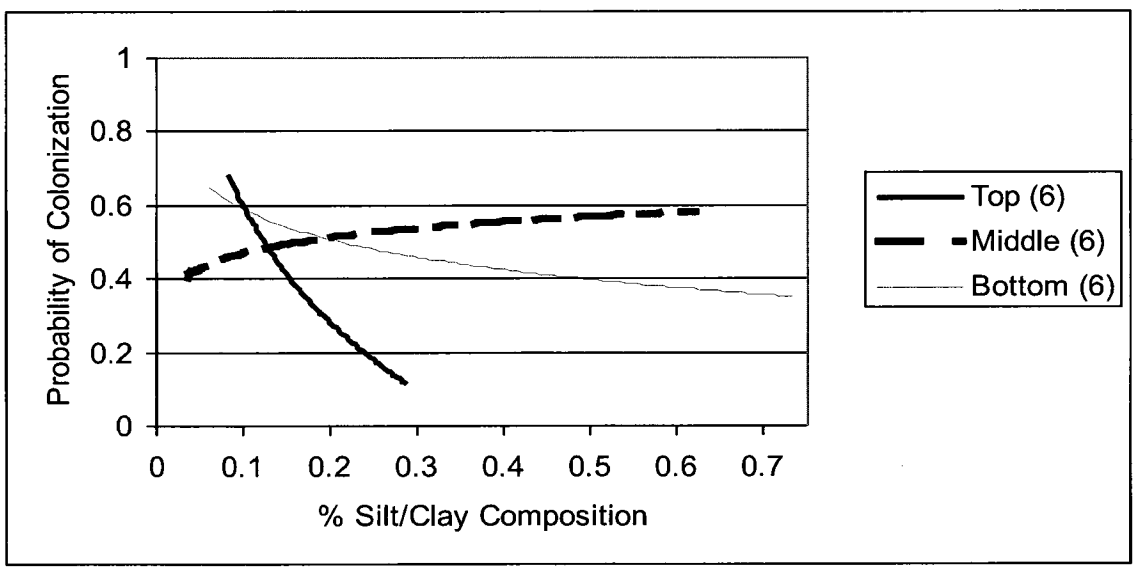

FIG. 23. Effect of silt and clay in three layers of soil: Top (0-30 cm), Middle (30-60 cm), and Bottom (60-90 cm) on the presence or absence of *T. septentrionalis* colonies in 2003. Numbers in parenthesis (*) indicate sample sizes.

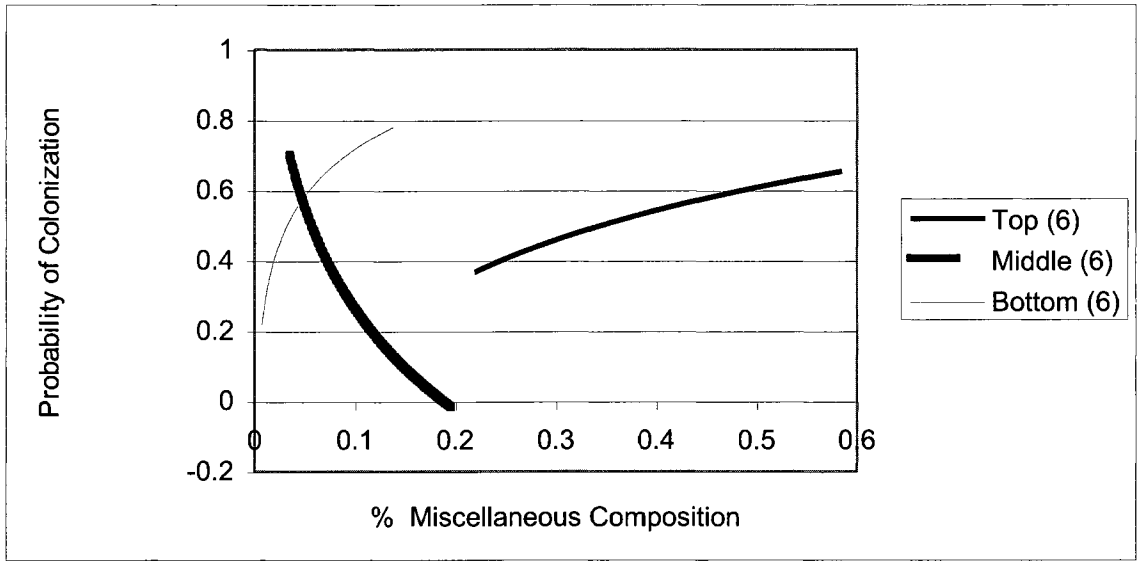

FIG. 24. Effect of miscellaneous materials in three layers of soil: Top (0-30 cm), Middle (30-60 cm), and Bottom (60-90 cm) on the presence or absence of *T. septentrionalis* colonies in 2003. Numbers in parenthesis (*) indicate sample sizes.

Cation Exchange Capacity

Cation exchange capacity, CEC, was measured during 2003 for populated and unpopulated areas (Table 8). No significant influence (B=0.004; S.E.=0.008; Wald=0.266; df=1; p=0.606; Exp(B)=1.004) could be attributed to the effect of CEC on the presence or absence of populations of *T. septentrionalis* (Figure 25). No significant differences were found between soil CEC at different levels or between levels and the presence or absence of *T. septentrionalis* populations.

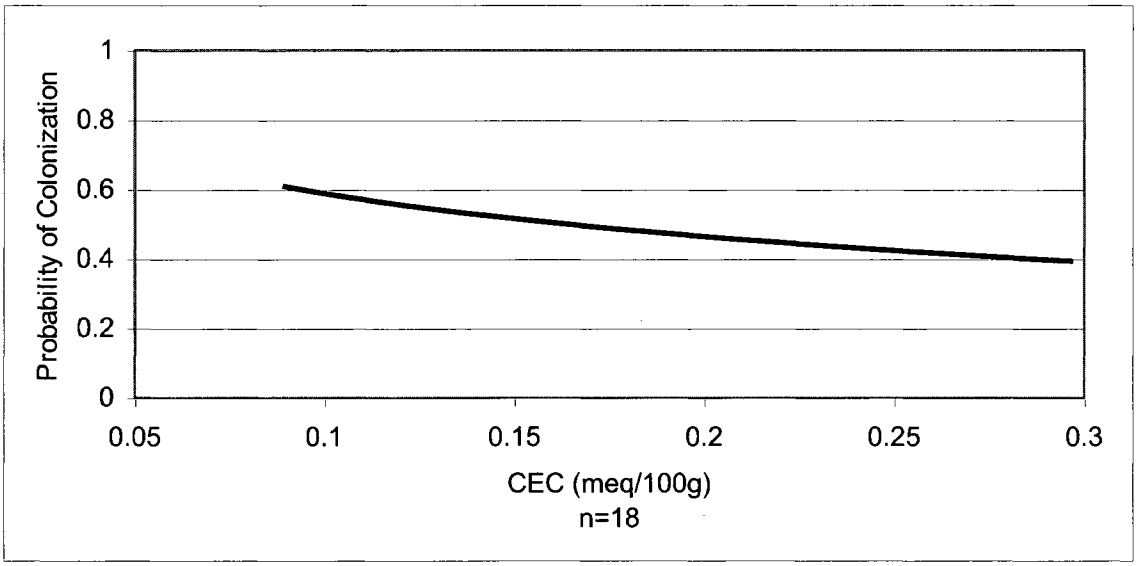

FIG. 25. Effect of CEC through all of the soil layers on the presence or absence of *T. septentrionalis* colonies in 2003.

pH

pH was measured over a two-year period (2003 and 2004), using different methods between years (Table 8). When pH and year of sampling were combined in a multifactor analysis, no significant difference (B=-0.975; S.E.=0.687; Wald=2.013; df=1; p=0.156; Exp(B)=0.377) was detected between populated and unpopulated areas (Figure 26), but these data are skewed by differences in sample sizes and sample depths between 2003 and 2004. The total pH and top soil layer were significantly different between 2003 and 2004: B=11.329; S.E.=1.657; Wald=46.745; df=1; p<0.001; Exp(B)=83180.729 and B=8.838; S.E.=1.689; Wald=27.395; df=1; p<0.001; Exp(B)=6890.229, respectively. When data from 2003 were analyzed independently from 2004, no significant difference (B=1.237; S.E.=1.447; Wald=0.731; df=1; p=0.393; Exp(B)=3.446) was found between populated and unpopulated sites. However, 2004 data did produce a significant difference (B=-14.181; S.E.=3.931; Wald=13.015; df=1;

$p<0.001$; $Exp(B)<0.001$) between areas populated by *T. septentrionalis* and unpopulated areas (Figure 27) for pH measured in the top 30 cm of soil.

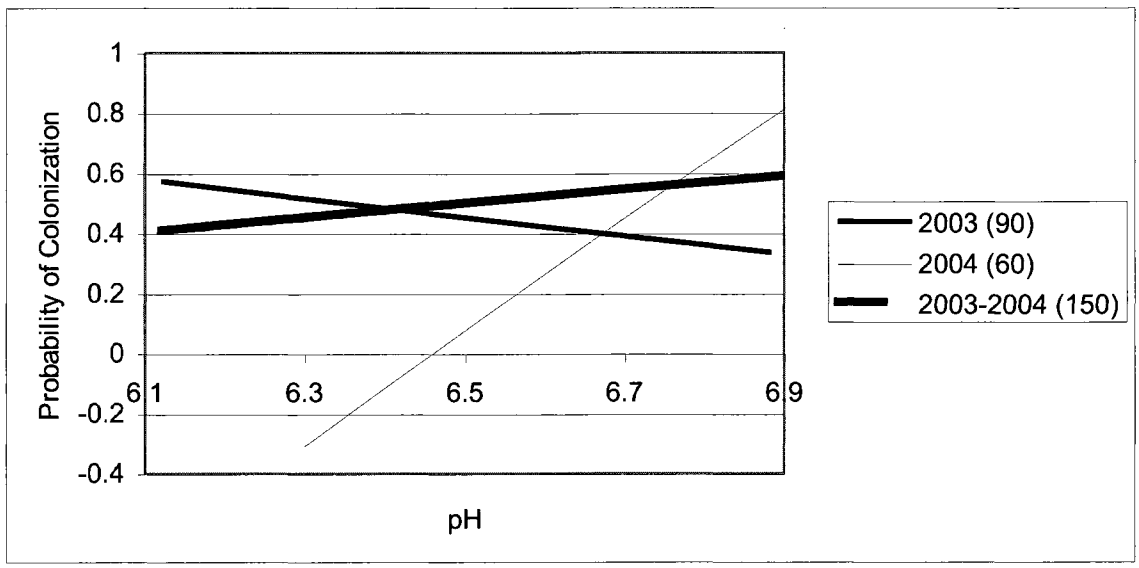

FIG. 26. Effect of pH in all soil layers of different study seasons on the presence, or absence, of *T. septentrionalis* colonies in 2003 and 2004. Numbers in parenthesis (*) indicate sample sizes.

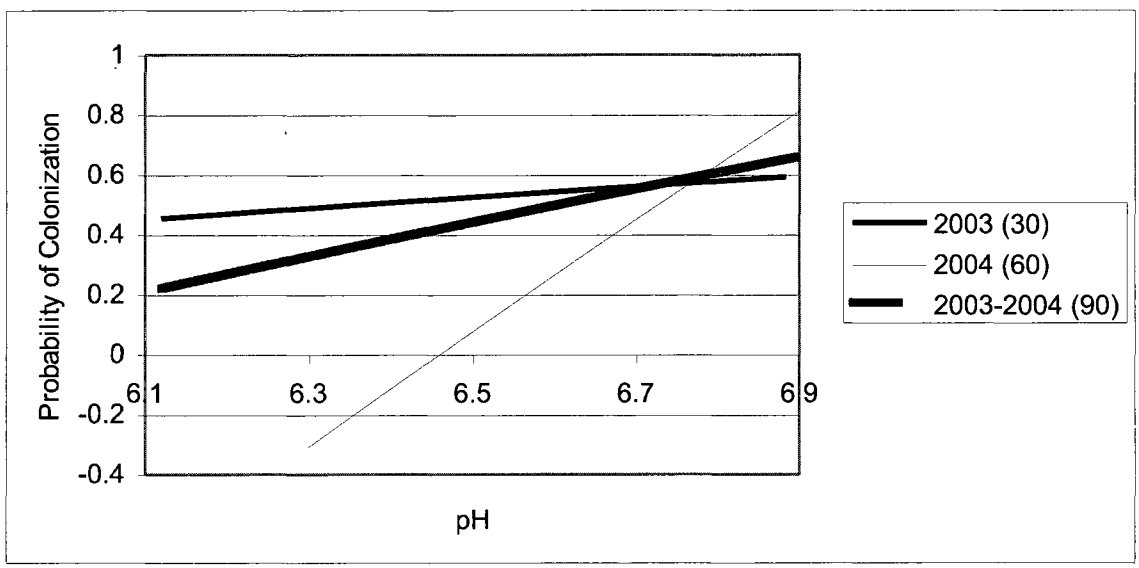

FIG. 27. Effect of pH in the first 30 cm of soil (Top) of different study seasons on the presence or absence of *T. septentrionalis* colonies in 2003 and 2004. Numbers in parenthesis (*) indicate sample sizes.

pH data from 2003 that were divided by soil level (Figure 28) produced no significant differences between populated and unpopulated areas for the top soil layer (B=-0.775; S.E.=1.929; Wald=0.161; df=1; p=0.688; Exp(B)=0.461), but when the data from 2003 and 2004 were combined a significant difference was found (B=-2.400; S.E.=0.955; Wald=6.320; df=1; p=0.012; Exp(B)=0.091). The pH of the middle soil layer in 2003 was also not significant (B=1.400; S.E.=2.872; Wald=0.238; df=1; p=0.626; Exp(B)=4.054), but a significant difference was found for the bottom layer (B=8.242; S.E.=4.104; Wald=4.034; df=1; p=0.045; Exp(B)=3796.591) in 2003.

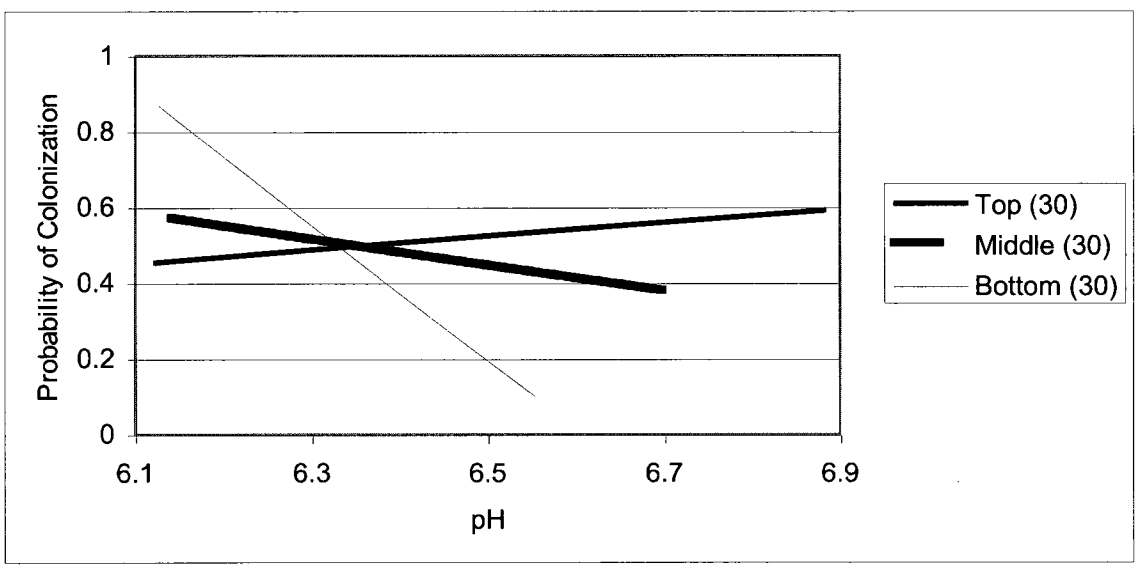

FIG. 28. Effect of pH in three layers of soil: Top (0-30 cm), Middle (30-60 cm), and Bottom (60-90 cm) on the presence, or absence, of *T. septentrionalis* colonies in 2003. Numbers in parenthesis (*) indicate sample sizes.

Organic Matter Composition

Data were collected for organic matter during only one year in 2003 (Table 8). Organic matter was significantly different between descending levels (top-middle: B=-5.538; S.E.=1.325; Wald=17.471; df=1; p<0.001; Exp(B)=0.004 and middle-bottm:

B=2.626; S.E.=0.929; Wald=7.997; df=1; p=0.005; Exp(B)=13.819), but was not significantly different between the *T. septentrionalis* populated and unpopulated areas (Figure 29) in the top (B=-1.774; S.E.=1.103; Wald=2.585; df=1; p=0.108; Exp(B)=0.170), middle (B=0.227; S.E.=1.241; Wald=0.034; df=1; p=0.855; Exp(B)=1.255), or bottom layer (B=-0.726; S.E.=1.159; Wald=0.393; df=1; p=0.531; Exp(B)=0.484). A combination of all the soil layers also did not produce a significant difference (B=-0.392; S.E.=0.434; Wald=0.817; df=1; p=0.366; Exp(B)=0.676) between populated and unpopulated sites.

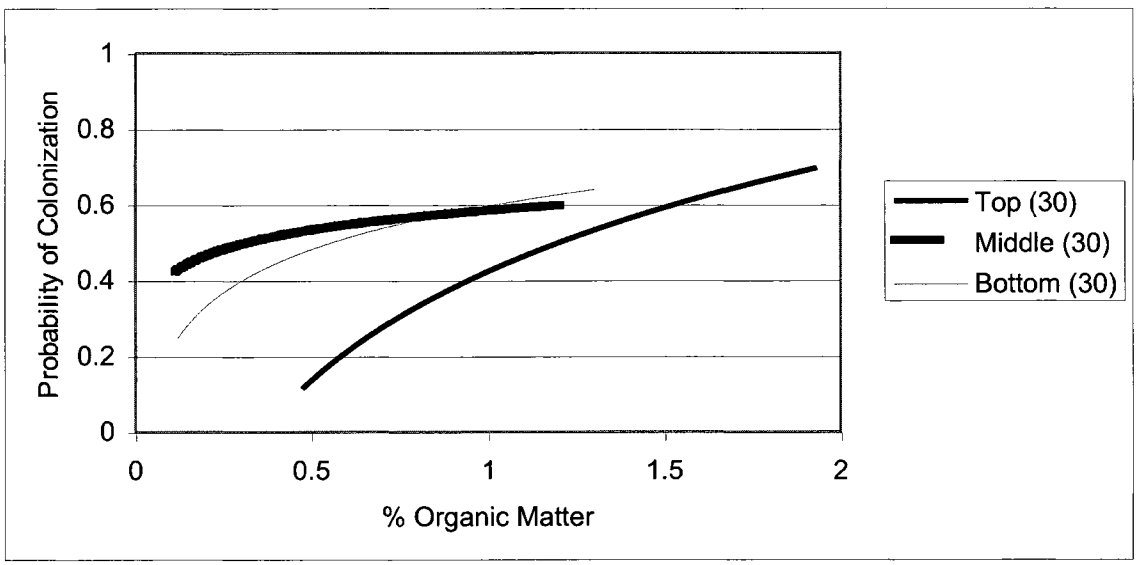

FIG. 29. Effect of organic matter materials in three layers of soil: Top (0-30 cm), Middle (30-60 cm), and Bottom (60-90 cm) on the presence or absence of *T. septentrionalis* colonies in 2003. Numbers in parenthesis (*) indicate sample sizes.

BIOTIC CONDITIONS

Nest Distribution

During the sampling periods of 2000 and 2001, data were collected from three sites to determine *T. septentrionalis* population densities (Table 9) and maps of the populations were constructed (Figures 30-33). The most populated study site was site B that contained 91 active nests in 2001, while site C contained the fewest with only 16 nests in 2000. Total area covered by the sites varied from a high of 844.34 m^2 for site B to a low of 124.22 m^2 for site A. When population densities were calculated, ranges were produced from 0.145 nests/m^2 in site A to 0.091 nests/m^2 for site C in 2000.

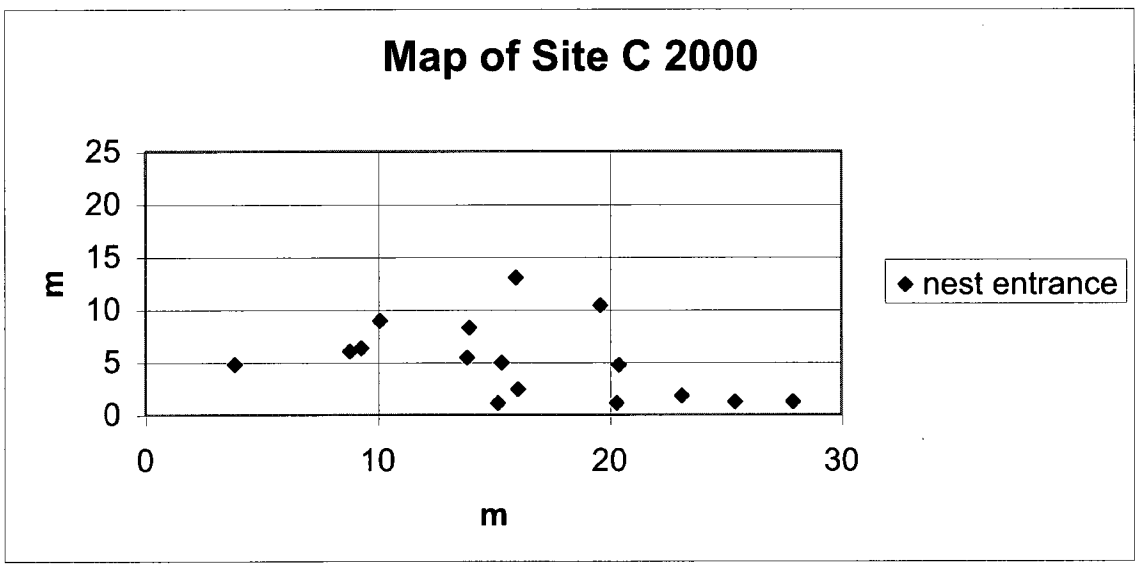

FIG. 30. Map of nest entrances identified as active during the 2000 study season in site C. Axes are denoted in meters.

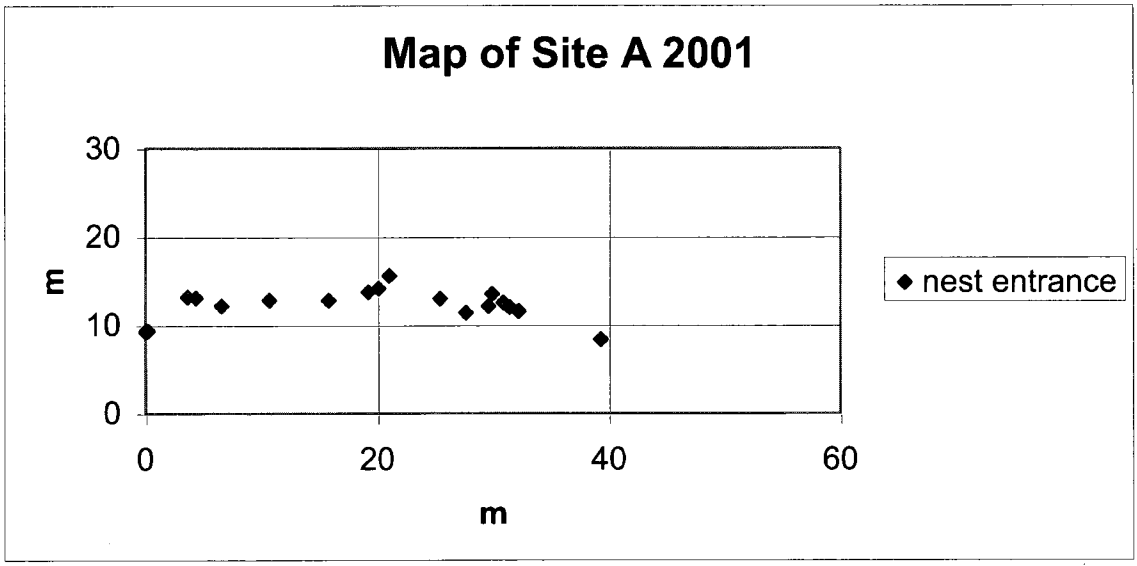

FIG. 31. Map of nest entrances identified as active during the 2001 study season in site A. Axes are denoted in meters.

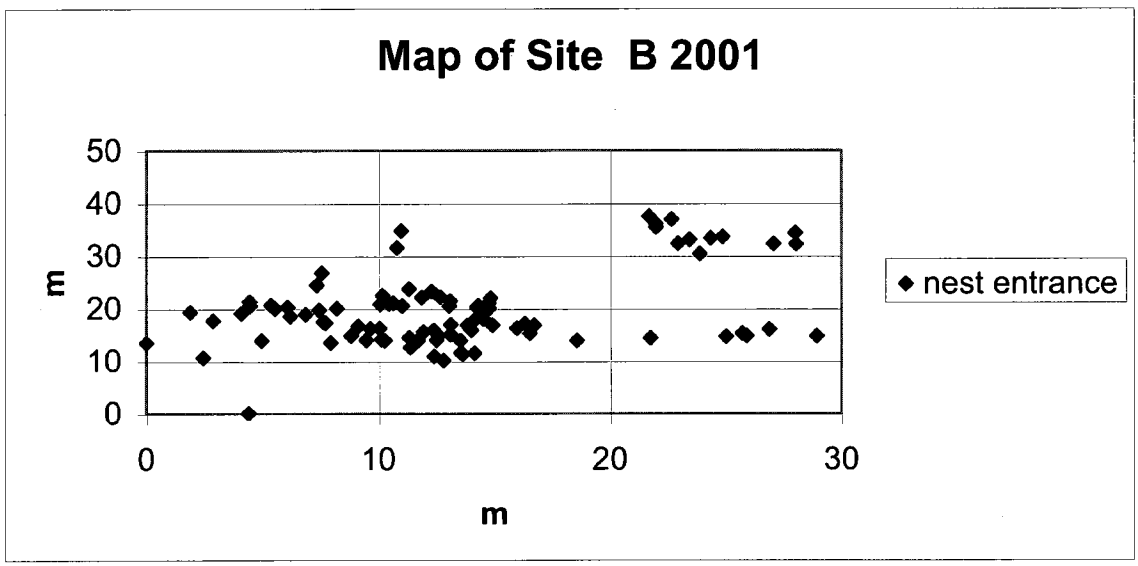

FIG. 32. Map of nest entrances identified as active during the 2001 study season in site B. Axes are denoted in meters.

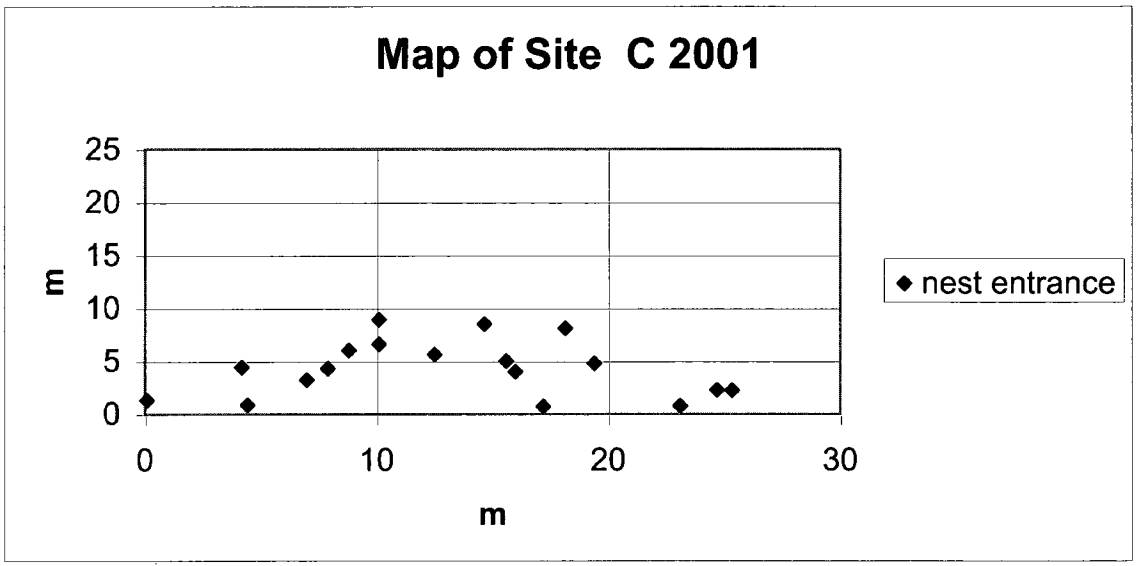

FIG. 33. Map of nest entrances identified as active during the 2001 study season in site C. Axes are denoted in meters.

TABLE 9. Population density data 2000-2001. Area was calculated inside a perimeter connecting the outermost entrances around the periphery of the population.

Site	# of Nests	Area (m^2)	Density (nests/ m^2)
C (2000)	16	175.1	0.091
A (2001)	18	124.2	0.145
B (2001)	91	844.3	0.108
C (2001)	18	136.8	0.132

Density data were analyzed in conjunction with the abiotic data collected during the same time period to detect possible links between the variables. Using a Pearson correlation coefficient with mean substitution for population densities, all abiotic variables were found to have a significant correlation with population densities except vegetative cover at the ground level ($r=0.385$; $p=0.123$), but there were not enough data to analyze variation in humidity. Vegetation cover at chest level ($r=-0.523$; $p<0.01$), soil temperatures ($r=-0.546$; $p<0.01$), and air temperatures ($r=-0.422$; $p<0.01$) where all found to be negatively correlated with *T. septentrionalis* densities. Light intensity levels

were also negatively correlated (r=-0.335; p=0.037) with population densities. When mean substitutions were not used for the density variable, no significant correlations were identified: ground level vegetation (r=-0.994; p=0.072), chest level vegetation (r=0.015; p=0.99), soil temperature (r=-0.282; p=0.718), air temperature (r=-0.148; p=0.852), and light intensity (r=0.996; p=0.057).

As with the correlation analysis, significant differences were detected for the abiotic factors between different population densities for all variables except vegetation cover at ground level (F=1.505; p=0.22) and humidity. Soil and air temperatures produced the greatest significant differences (F=20.085; p<0.001 and F=9.969; p<0.001, respectively) between population densities, and mean temperatures ranged from 19.06°C in site B to 26.9°C in site C (2000) for soil and 21.0°C in site B to 27.3°C in site C (2000) for air. Vegetative cover at chest levels ranged from 33.5% in site C (2001) to 61.6% in site A (F=7.003; p=0.001). A significance of p=0.02 (F=3.628) was calculated for light intensities ranging from 62.4 in site B to 558.1 lumen in site A.

At the end of 2001, a new site (D) was located. Nests in this site, as well as the previously studied sites, were marked and observed during the 2002 study period to determine whether they were still active (Figures 34-37). In 2002, numbers of nests in each site ranged from 36 to 150 and spanned areas from 253.9 m^2 to 2081.9 m^2 (Table 10).

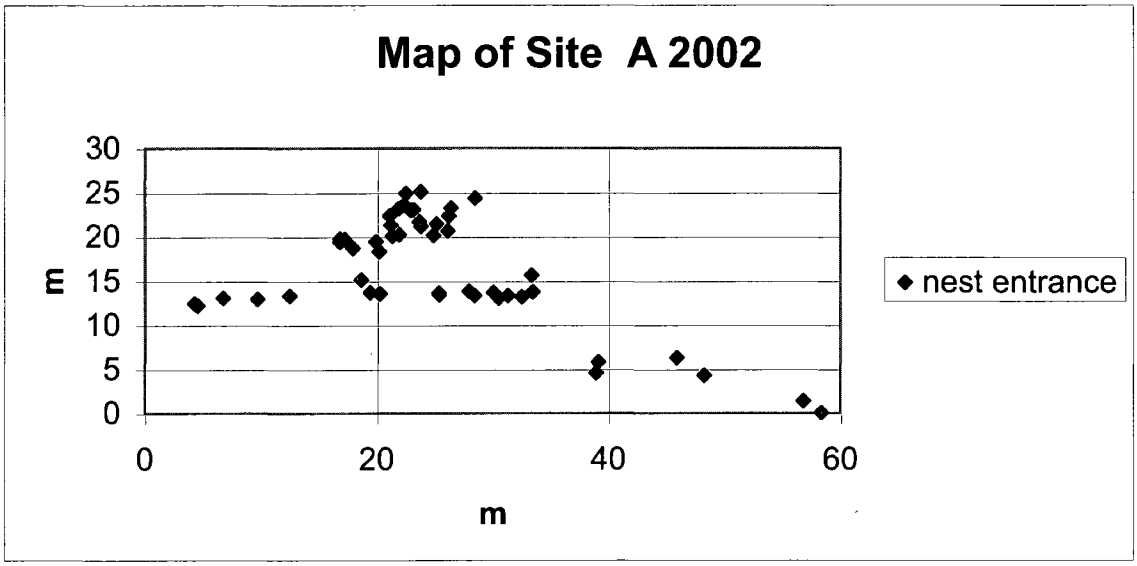

FIG. 34. Map of nest entrances identified as active during the 2002 study season in site A. Axes are denoted in meters.

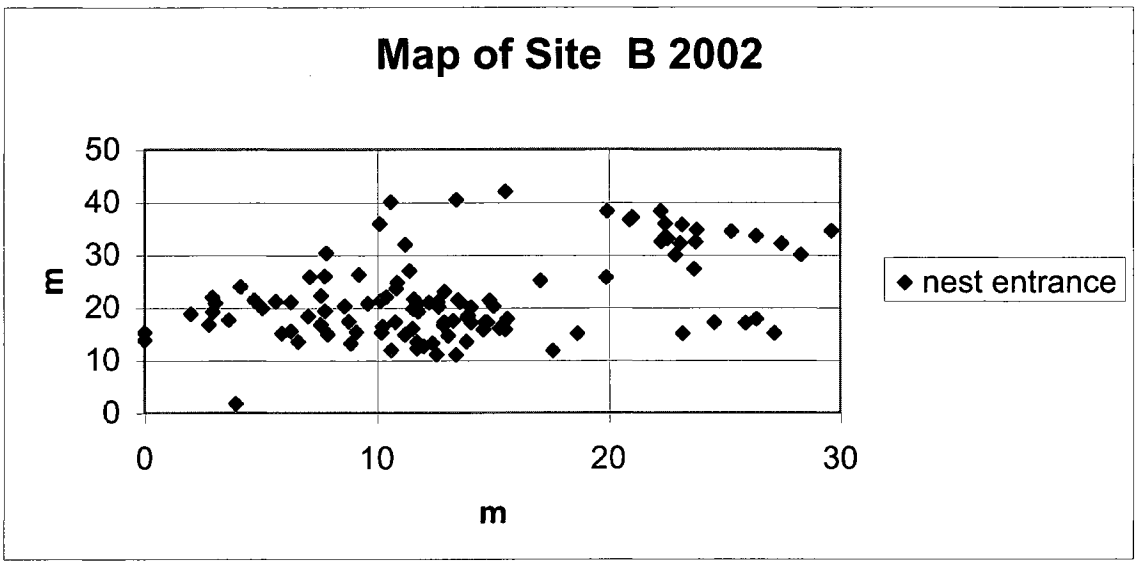

FIG. 35. Map of nest entrances identified as active during the 2002 study season in site B. Axes are denoted in meters.

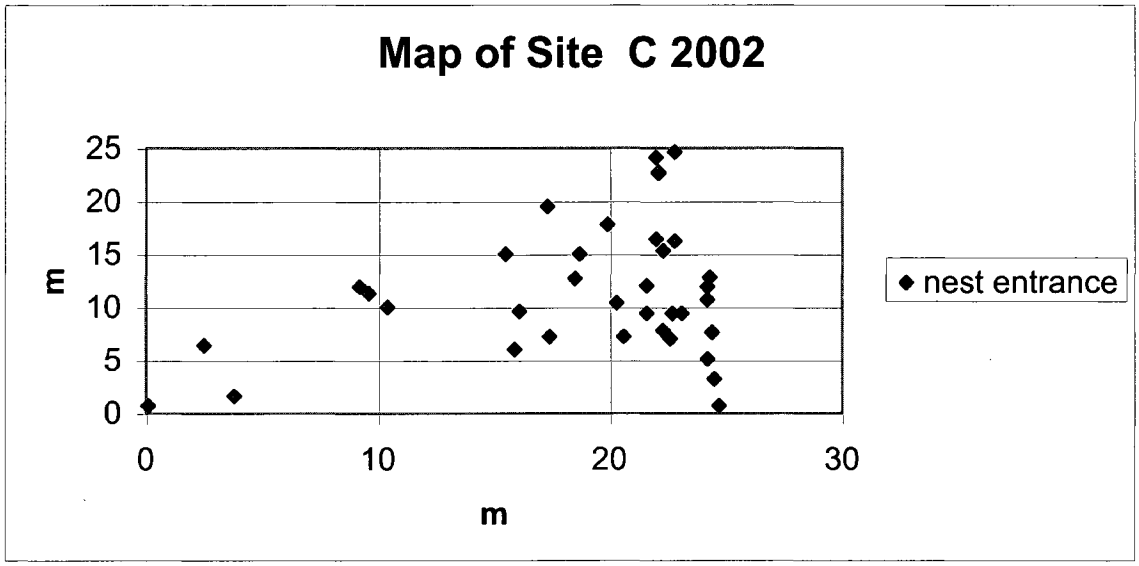

FIG. 36. Map of nest entrances identified as active during the 2002 study season in site C. Axes are denoted in meters.

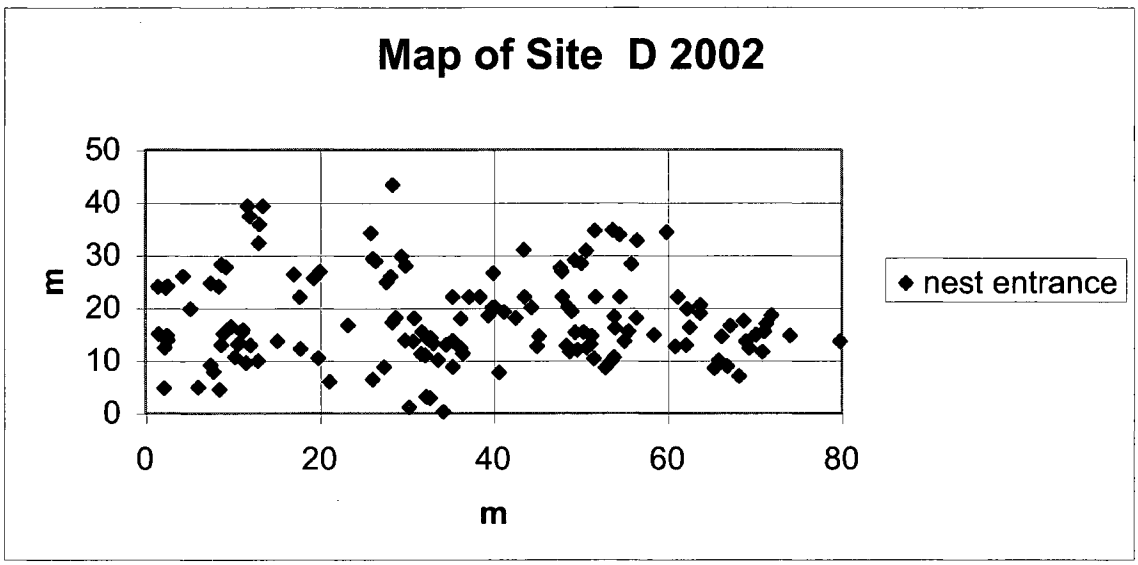

FIG. 37. Map of nest entrances identified as active during the 2002 study season in site D. Axes are denoted in meters.

TABLE 10. Population density data 2002. Area was calculated inside a perimeter connecting the outermost entrances around the periphery of the population.

Site	# of Nests	Area (m^2)	Density (nests/ m^2)
A (2002)	48	462.4	0.104
B (2002)	108	819.1	0.132
C (2002)	36	253.9	0.142
D (2002)	150	2081.9	0.072

Ant Community Structure

The total number of ant species collected during the study was not significantly different (B=-0.023; S.E.=0.028; Wald=0.672; df=1; p=0.412; Exp(B)=1.023) in the presence (27) or absence (30) of *T. septentrionalis* (Figure 38). Analyses using Shannon's Diversity and Evenness Indices indicate that areas populated by *T. septentrionalis* are less diverse (2.47 versus 2.61) and less even (0.75 versus 0.77) than unpopulated areas. In areas populated by *T. septentrionalis*, ant species representing five subfamilies were identified; only four subfamilies were represented in unpopulated areas (Table 11). A total of 27 ant species were identified in areas populated by *T. septentrionalis;* 30 ant species were identified in unpopulated areas (Table 12). The most pervasive species were *Pheidole morrisi* and *Solenopsis* sp., which were the most abundant species in both treatment and control areas. Only *Aphaenogaster treatae* was found to be significantly higher (B=-0.594; S.E.=0.247; Wald=5.794; df=1; p=0.016; Exp(B)=0.552) in treatments not populated by *T. septentrionalis* than in areas populated by *T. septentrionalis* (Figure 39). Some species occurred in low numbers in either the populated or unpopulated site, but not both (Table 12).

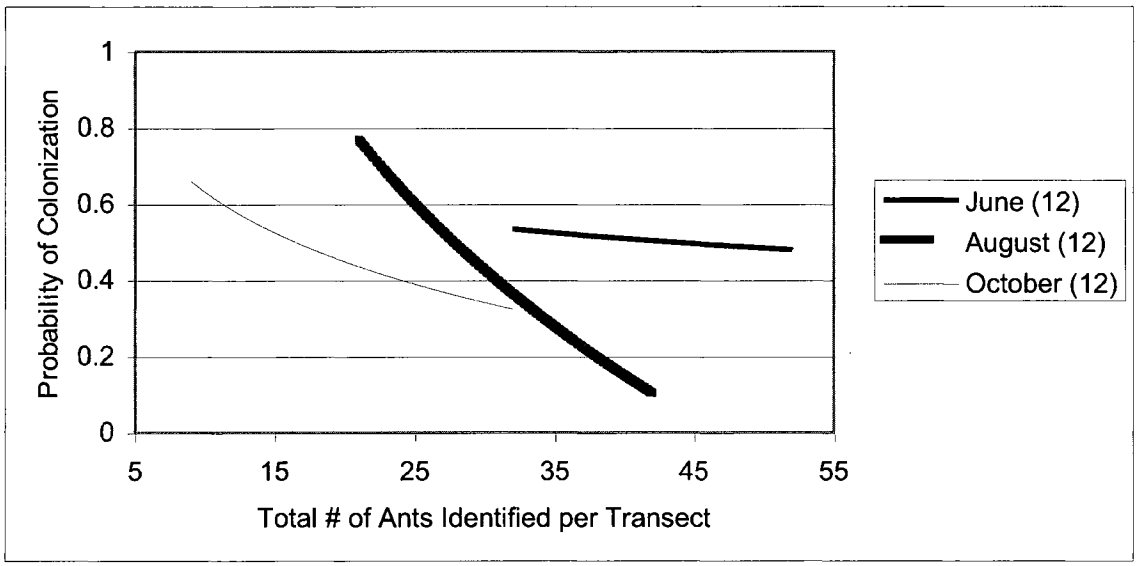

FIG. 38. Effect of total ant community composition for three collection dates on the presence, or absence, of *T. septentrionalis* colonies. Numbers in parenthesis (*) indicate sampled transects.

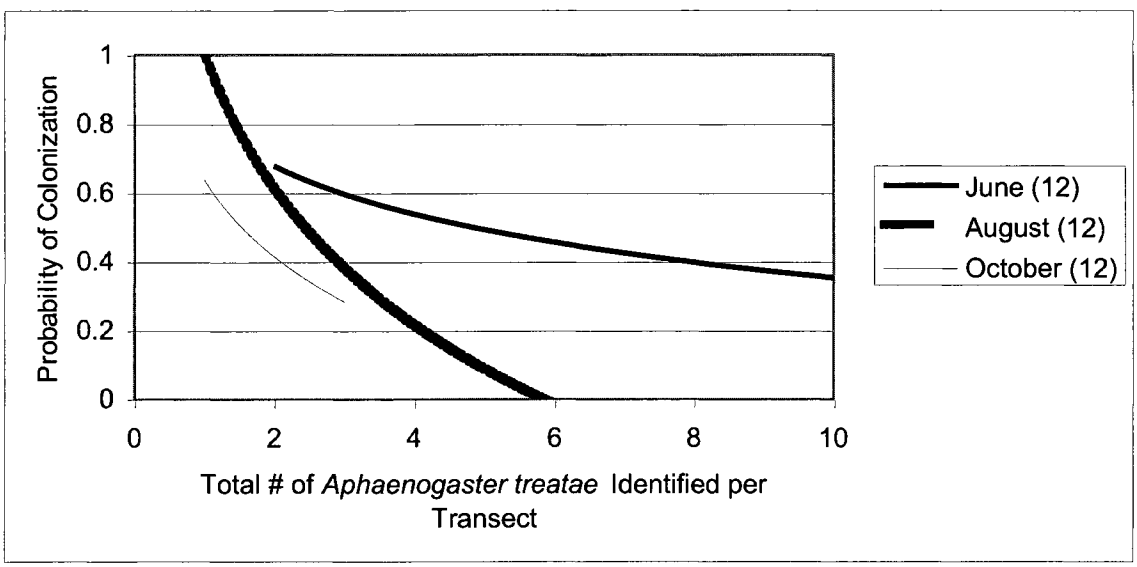

FIG. 39. Effect of *Aphaenogaster treatae* for three collection dates on the presence, or absence, of *T. septentrionalis* colonies. Numbers in parenthesis (*) indicate sampled transects.

The month of capture (Table 12) had a significant influence on the total number of captured individuals (Figure 38), subfamilies (Formicinae, Myrmicinae, and Dolichoderinae) (Figures 40-42), and individual species *A. treatae* (Figure 39) and *Solenopsis* sp. (Figure 43). The total number of ants and Myrmicinae captured significantly differed in successive months (total: June-August (B=-0.344; S.E.=0.135; Wald=6.510; df=1; p=0.011; Exp(B)=0.709) and August-October (B=-0.287; S.E.=0.125; Wald=5.247; df=1; p=0.022; Exp(B)=0.750); Myrmicinae: June-August (B=-0.426; S.E.=0.167; Wald=6.507; df=1; p=0.011; Exp(B)=0.653) and August-October (B=-0.384; S.E.=0.168; Wald=5.221; df=1; p=0.022; Exp(B)=0.681). The subfamilies Formicinae and Dolichoderinae both exhibited significantly higher numbers in June than August and October (Formicinae: June-August (B=-0.749; S.E.=0.326; Wald=5.287; df=1; p=0.021; Exp(B)=0.473) and June-October (B=-1.312; S.E.=0.501; Wald=6.860; df=1; p=0.009; Exp(B)=0.269); Dolichoderinae: June-August (B=-0.260; S.E.=0.122; Wald=4.494; df=1; p=0.034; Exp(B)=0.771) and June-October (B=-0.771; S.E.=0.301; Wald=6.537; df=1; p=0.011; Exp(B)=0.463). The number of captured individuals of *A. treatae* was significantly higher in June than in August (B=-0.456; S.E.=0.223; Wald=4.191; df=1; p=0.041; Exp(B)=0.634) and October (B=-1.549; S.E.=0.697; Wald=4.934; df=1; p=0.026; Exp(B)=0.212), and both June (B=-1.565; S.E.=0.655; Wald=5.720; df=1; p=0.017; Exp(B)=0.209)and August (B=-1.047; S.E.=0.402; Wald=6.776; df=1; p=0.009; Exp(B)=0.351) had a significantly higher number of individuals identified for *Solenopsis* sp. than did October.

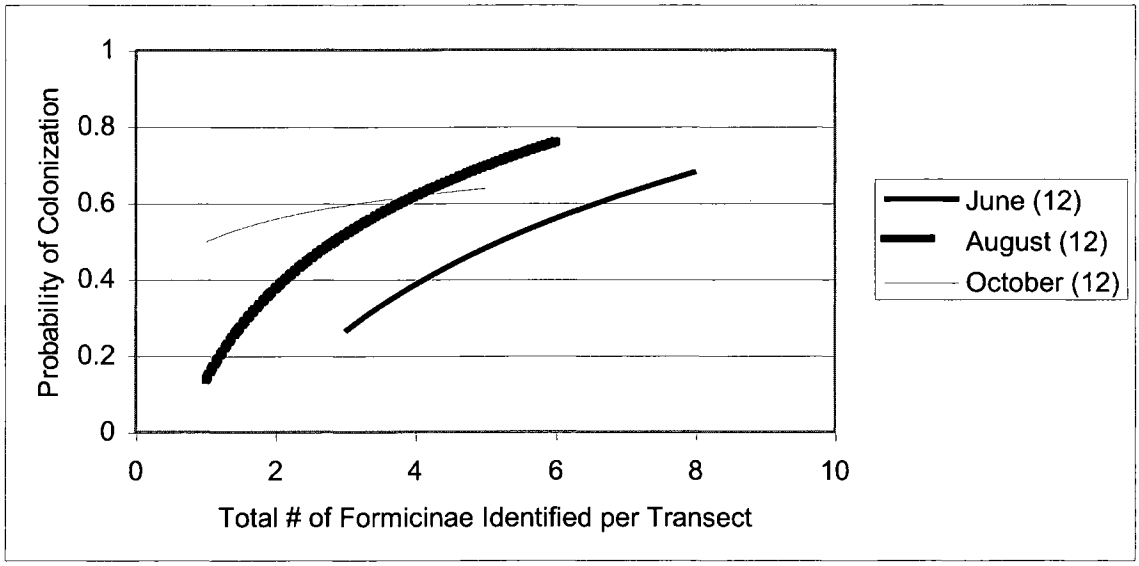

FIG. 40. Effect of Formicinae community for three collection dates on the presence, or absence, of *T. septentrionalis* colonies. Numbers in parenthesis (*) indicate sampled transects.

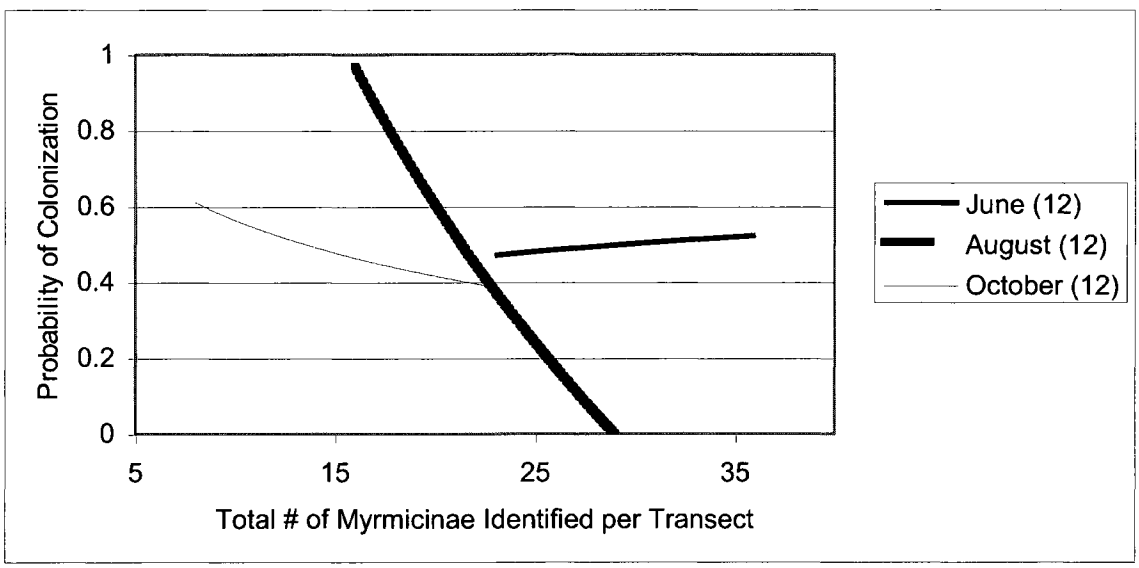

FIG. 41. Effect of Myrmicinae community for three collection dates on the presence, or absence, of *T. septentrionalis* colonies. Numbers in parenthesis (*) indicate sampled transects.

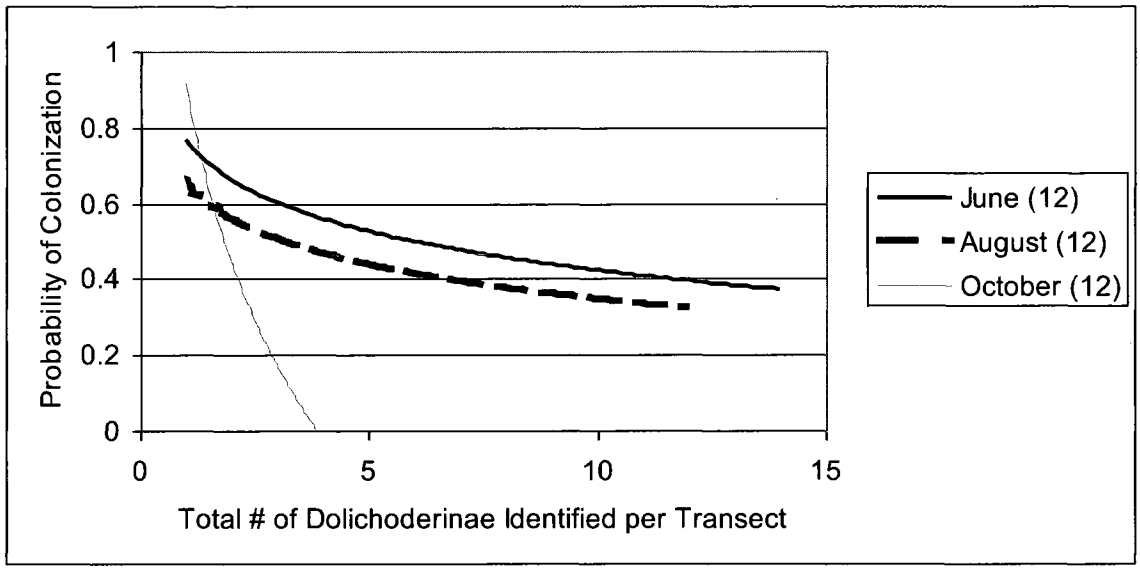

FIG. 42. Effect of Dolichoderinae community for three collection dates on the presence, or absence, of *T. septentrionalis* colonies. Numbers in parenthesis (*) indicate sampled transects.

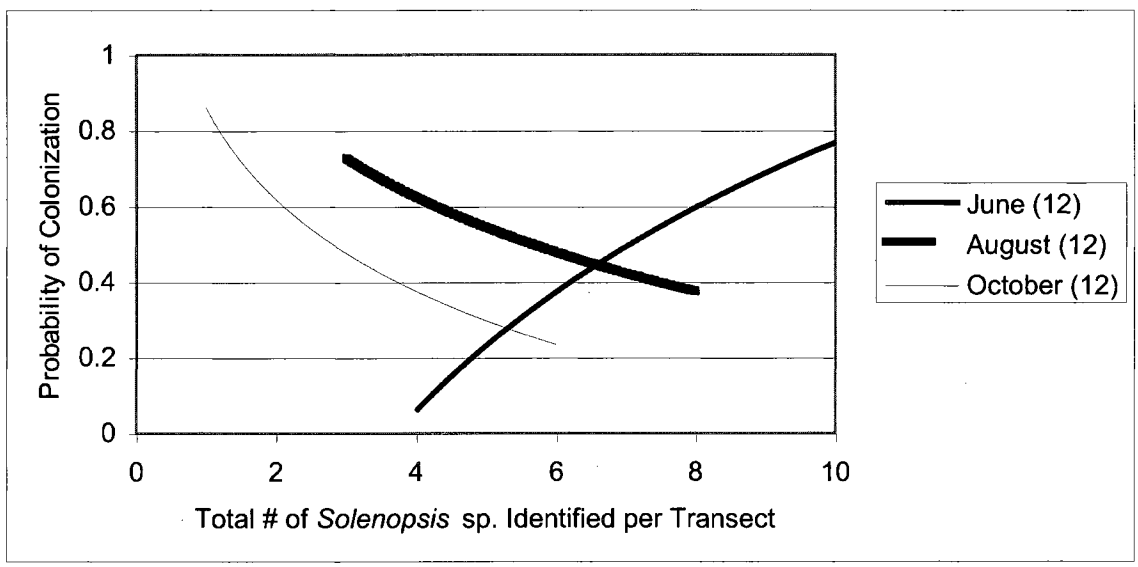

FIG. 43. Effect of *Solenopsis* sp. for three collection dates on the presence, or absence, of *T. septentrionalis* colonies. Numbers in parenthesis (*) indicate sampled transects.

TABLE 11. Effect of ant subfamilies and species on presence or absence of *T. septentrionalis* colonies. Values are the total numbers of individual ants collected per transect within populated and unpopulated areas during the three collection months. "None" values indicate traps with no ants present. An individual ant is defined as a single species occurring in a single pitfall trap. Values in parenthesis (*) indicate the number of individual species identified for each subfamily.

Month	Treatment	Formicinae	Myrmicinae	Dolichoderinae	Ponerinae	Ecitoninae	None	Total
June	Populated	35 (6)	180 (13)	37 (2)	6 (1)	0	0	258 (22)
	Unpopulated	31 (8)	180 (12)	50 (2)	1	0	1	262 (23)
August	Populated	23 (4)	113 (12)	12 (2)	2 (1)	2 (1)	2	152 (20)
	Unpopulated	17 (4)	148 (14)	25 (3)	0	0	2	190 (21)
October	Populated	13 (4)	79 (9)	3 (1)	1	0	6	96 (15)
	Unpopulated	10 (3)	92 (11)	11 (2)	1	0	8	114 (17)
Total	Populated	71 (8)	372 (15)	52 (2)	9 (1)	2 (1)	8	506 (27)
	Unpopulated	58 (10)	420 (15)	86 (3)	2 (2)	0	11	566 (30)

TABLE 12. Lists of species identified in the areas that are populated or not populated by *T. septentrionalis*. Bolded subtitles indicate subfamilies followed by representative species. Letters in parenthesis (*) indicate the months of capture for each species: J = June, A = August, and O = October.

POPULATED AREAS	UNPOPULATED AREAS
Formicinae	**Formicinae**
Acanthomyops interjectus (J)	*Brachymyrmex depilis (J, O)*
Brachymyrmex depilis (J, A, O)	*Camponotus americanus* (A)
Camponotus americanus (J, O)	*Formica pallidefulva* (J, A, O)
Formica pallidefulva (J, A, O)	*Formica subsericea* (J)
Lasius flavus (J)	*Lasius alienus* (J)
Paratrechina arenivaga (A)	*Paratrechina arenivaga* (J, A)
Paratrechina parvula (J, A, O)	*Paratrechina faisonensis* (J)
Prenolepis imparis (O)	*Paratrechina parvula* (J, A, O)
	Paratrechina vividula (J)
	Prenolepis imparis (O)
Myrmicinae	**Myrmicinae**
Aphaenogaster floridana (A)	*Aphaenogaster floridana* (A)
Aphaenogaster rudis (J, A)	*Aphaenogaster rudis* (J, A, O)
Aphaenogaster treatae (J, A, O)	*Aphaenogaster treatae* (J, A, O)
Crematogaster ashmeadi (J, A)	*Crematogaster ashmeadi* (J, A, O)
Crematogaster lineolata (J)	*Crematogaster lineolata* (J, A, O)
Crematogaster pilosa (J, A)	*Crematogaster pilosa* (J, A)
Leptothorax davisi (J, A, O)	*Leptothorax davisi* (J, A, O)
Leptothorax pergandei (J, A)	*Leptothorax pergandei* (J, A, O)
Myrmecina americana (J, O)	*Myrmecina americana* (A)
Myrmica pinetorum (A, O)	*Myrmica pinetorum* (J, A, O)
Pheidole bicarinata (J, O)	*Pheidole bicarinata* (J, A, O)
Pheidole davisi (J, A, O)	*Pheidole davisi* (J, A, O)
Pheidole morrisi (J, A, O)	*Pheidole morrisi* (J, A, O)
Solenopsis sp. (J, A, O)	*Pyramica clypeata* (A)
Trachymyrmex septentrionalis (J, A, O)	*Solenopsis* sp. (J, A, O)
Dolichoderinae	**Dolichoderinae**
Forelius pruinosus (J, A)	*Dorymyrmex bureni* (A, O)
Tapinoma sessile (J, A, O)	*Forelius pruinosus* (J, A)
	Tapinoma sessile (J, A, O)
Ponerinae	**Ponerinae**
Hyponera opacior (J, A, O)	*Hyponera opacior* (O)
	Ponera pennsylvanica (J)
Ecitoninae	
Neivamyrmex texanus (A)	

Vegetation Analysis

Plant species were divided into ground and canopy vegetation for more detailed analyses (Table 13). Ground vegetation (Figure 44) was not found to be significantly different between populated and unpopulated areas during the two-year span of the study (B=0.000; S.E.=0.000; Wald=0.045; df=1; p=0.832; Exp(B)=1.000), and no significantly different was found when individual years were considered. Canopy vegetation (Figure 45) demonstrated no significant differences between treatments at any time (B=0.000; S.E.=0.018; Wald=0.000; df=1; p=1.000; Exp(B)=1.000). *Opuntia compressa* and *Sassafras albidum* were both identified within populated areas during the foraging portion of the study (see below), but neither were located within the quadrants established during the vegetation analysis. In total, 15 species of plants were identified in areas populated by *T. septentrionalis*, while 22 species were identified in unpopulated areas. Shannon's Diversity Index demonstrated that areas populated by *T. septentrionalis* were less diverse (1.37 versus 2.13) and less even (0.51 versus 0.69) than those areas that were unpopulated by *T. septentrionalis*.

TABLE 13. Mean number of individual plants observed during a two-year period and calculated to produce an expected observation per 10000m^2. Values in parenthesis (*) indicate the number of different species identified.

Treatment	Populated		Unpopulated	
Year of Survey	2006	2007	2006	2007
Andropogon virginicus	8.3	0.0	50.0	22.2
Arundinaria gigantea	75.0	0.0	0.0	33.3
Carex rosea	0.0	0.0	0.0	11.1
Gaylussacia baccata	116.7	22.2	425.0	544.4
Gelsemium sempervirens	0.0	66.7	75.0	0.0
Kalmia angustifolia	108.3	100.0	108.3	33.3
Lindera benzoin	0.0	0.0	8.3	0.0
Liquidambar styraciflua	0.0	0.0	8.3	0.0
Panicum lancearium	25.0	44.4	400.0	44.4
Pinus sp.	7.5 (2)	16.7 (1)	10.0 (2)	6.7 (2)
Polygonella polygama	0.0	0.0	500.0	0.0
Pteridium aquilinum	350.0	200.0	350.0	444.4
Pyxidanthera barbulata (%)	0.017	0.0	0.071	0.0056
Quercus sp.	30.0 (2)	3.3 (1)	32.5 (2)	6.7 (1)
Sassafras albidum[1]	0.0	0.0	16.7	0.0
Smilax rotundifolia	208.3	77.8	25.0	144.4
Solidago erecta	0.0	22.2	0.0	0.0
Sphagnum perichaetiale (%)	0.0	0.0	0.0083	0.078
Vaccinium sp	1141.7 (1)	2200.0 (1)	791.7 (1)	677.8 (2)

[1]-Foraged by *T. septentrionalis* but not identified in the vegetation survey.

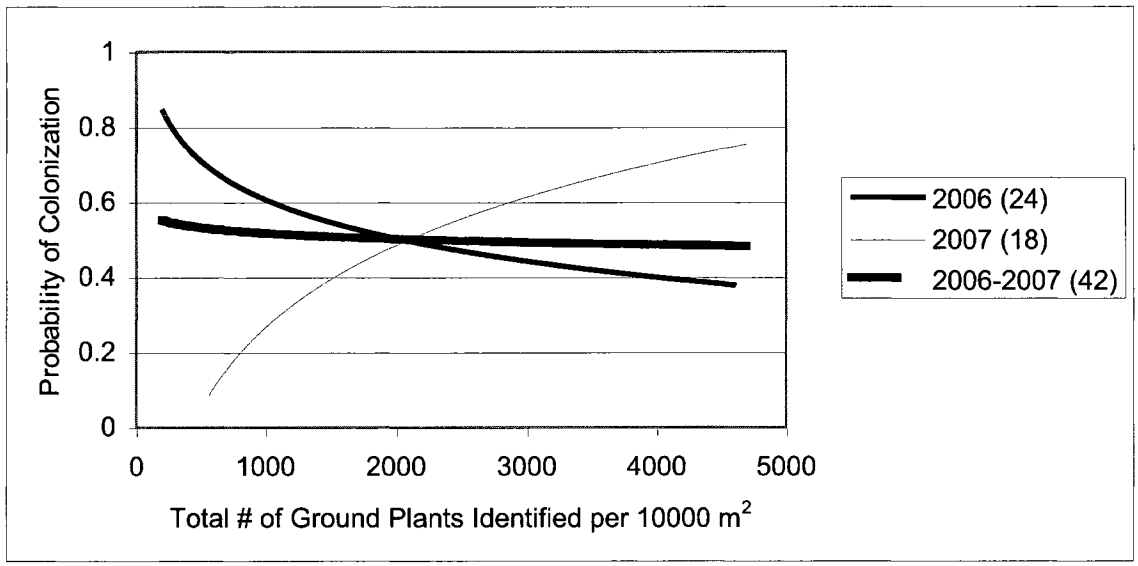

FIG. 44. Effect of ground vegetation community composition on the presence, or absence, of *T. septentrionalis* colonies in 2006 and 2007. Numbers in parenthesis (*) indicate sample sizes.

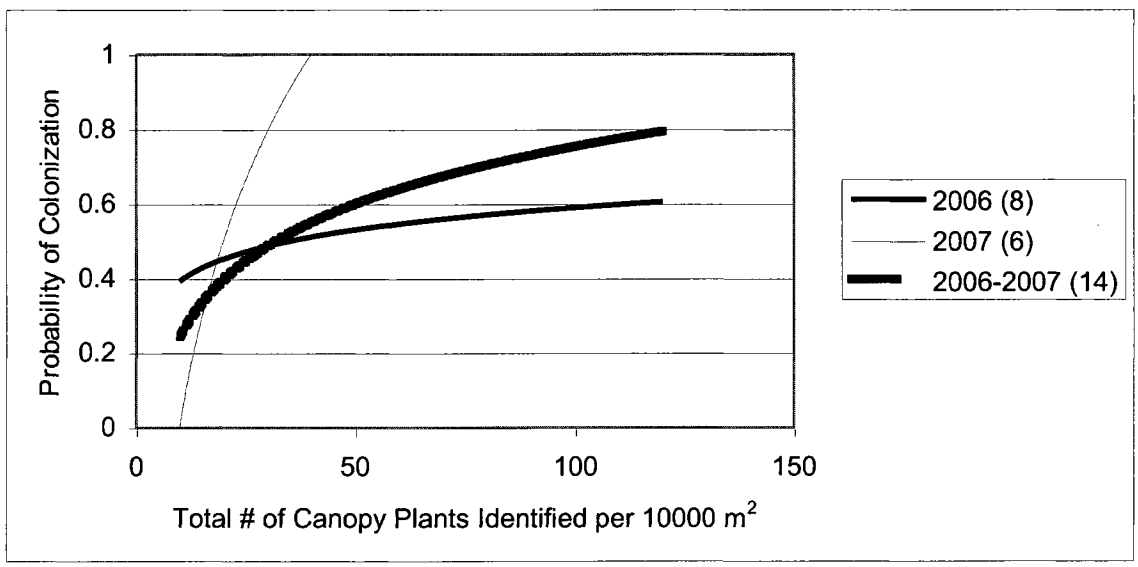

FIG. 45. Effect of canopy vegetation community composition on the presence, or absence, of *T. septentrionalis* colonies in 2006 and 2007. Numbers in parenthesis (*) indicate sample sizes.

In both years, *Gaylussacia baccata* was significantly more abundant (B=0.004; S.E.=0.002; Wald=6.753; df=1; p=0.009; Exp(B)=1.004) with 484.7 plants in the control areas without *T. septentrionalis* than the 69.5 plants in populated areas (Figure 46). Analyses of individual years produced the same significant relation between populated and unpopulated areas in 2006 (B=0.004; S.E.=0.002; Wald=4.255; df=1; p=0.039; Exp(B)=1.004) but not 2007 (B=0.005; S.E.=0.005; Wald=1.240; df=1; p=0.265; Exp(B)=1.005) for *G. baccata*. Two species represented the genus *Vaccinium*. The *Vaccinium* sp. numbers are dominated by *Vaccinium pallidum* that were analyzed individually (Figures 47 and 48). *Vaccinium* sp. (B=-0.001; S.E.=0.000; Wald=4.548; df=1; p=0.033; Exp(B)=0.999) and *V. pallidum* (B=-0.001; S.E.=0.000; Wald=4.685; df=1; p=0.030; Exp(B)=0.999) numbers were significantly higher in areas populated by *T. septentrionalis* than unpopulated areas over the full two-year sampling period of the study and 2007 individually (*Vaccinium* sp. : B=-0.001; S.E.=0.000; Wald=4.009; df=1; p=0.045; Exp(B)=0.999 and *V. pallidum*: B=-0.001; S.E.=0.000; Wald=4.136; df=1; p=0.042; Exp(B)=0.999) .

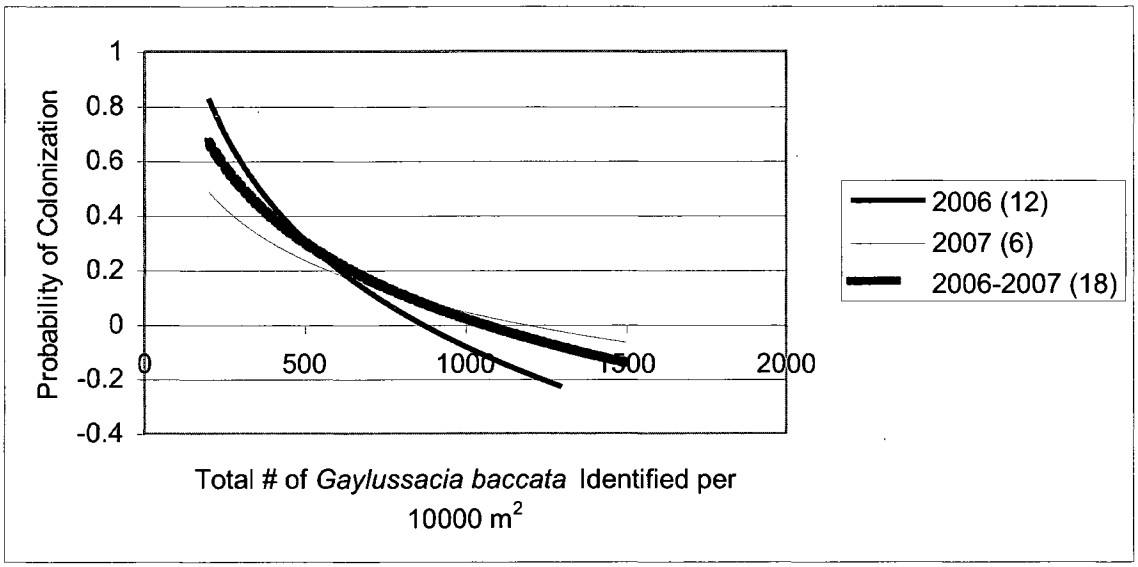

FIG. 46. Association of *Gaylussacia baccata* with the presence, or absence, of *T. septentrionalis* colonies in 2006 and 2007. Numbers in parenthesis (*) indicate sample sizes.

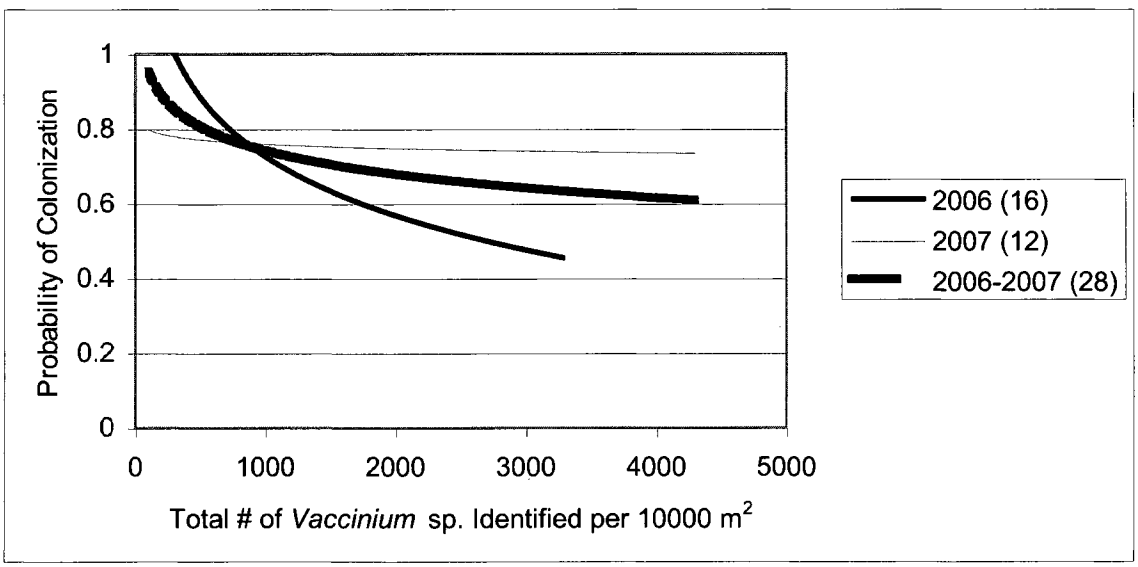

FIG. 47. Association of *Vaccinium* sp. populations with the presence, or absence, of *T. septentrionalis* colonies in 2006 and 2007. Numbers in parenthesis (*) indicate sample sizes.

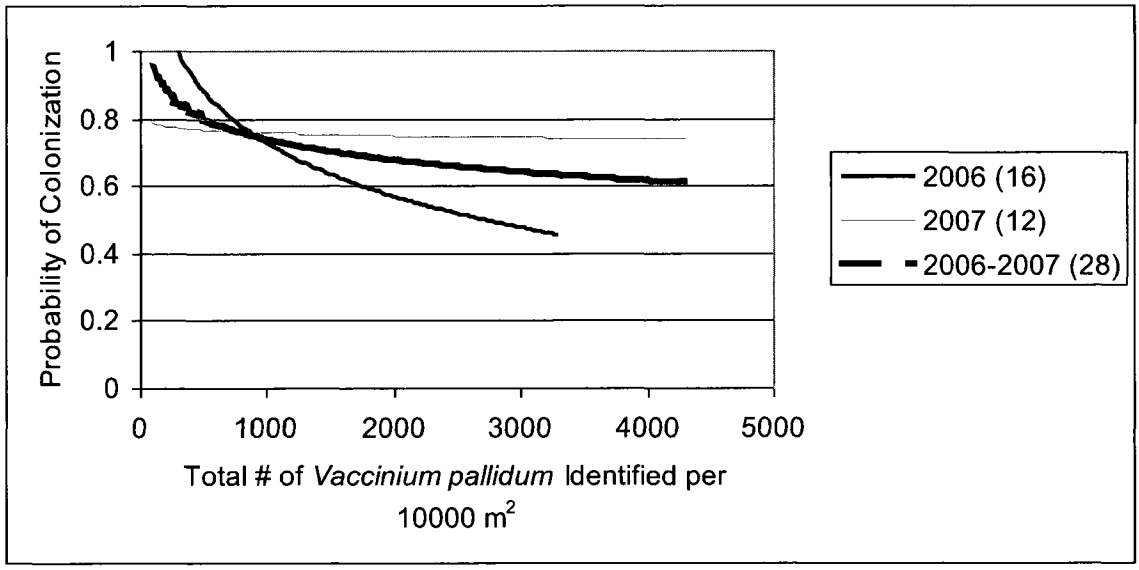

FIG. 48. Association of *Vaccinium pallidum* with the presence, or absence, of *T. septentrionalis* colonies in 2006 and 2007. Numbers in parenthesis (*) indicate sample sizes.

Foraging Analysis

During the foraging collection period, 12 different types of forage materials were identified (Table 14), with the largest totals being produced by various berry species (*Vaccinium* sp. or *Gaylussacia baccata*). Air temperatures (Figures 49-52) significantly influenced forage collection rates for the total pieces of forage collected ($r=0.168$; $p=0.014$; $n=215$), the number of pieces of berry collected ($r=0.226$; $p=0.047$; $n=78$), the number of *Quercus* sp. leaf fragments collected ($r=-0.347$; $p=0.038$; $n=36$), and the number of *Pteridium aquilinum* pieces collected ($r=0.369$; $p=0.012$; $n=46$). Ground temperature was correlated with air temperature ($r=0.448$; $p<0.001$; $n=215$), but only *Quercus* sp. leaf collection (Figure 53) correlated with ground temperature ($r=-0.524$; $p=0.001$; $n=36$).

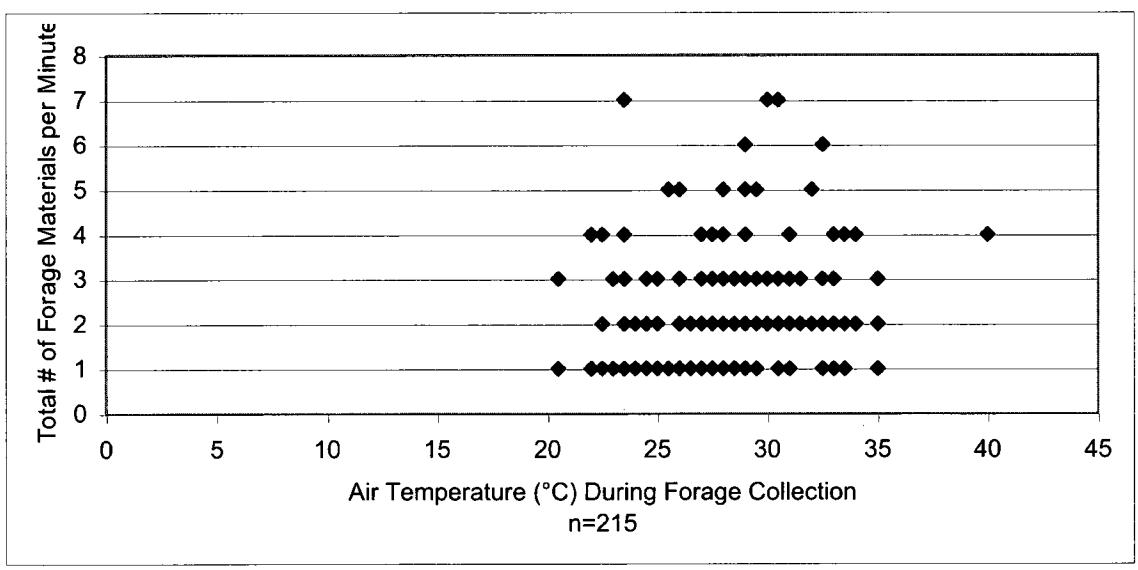

FIG. 49. Effect of air temperature on the numbers of forage material collected by nests during a one minute time period.

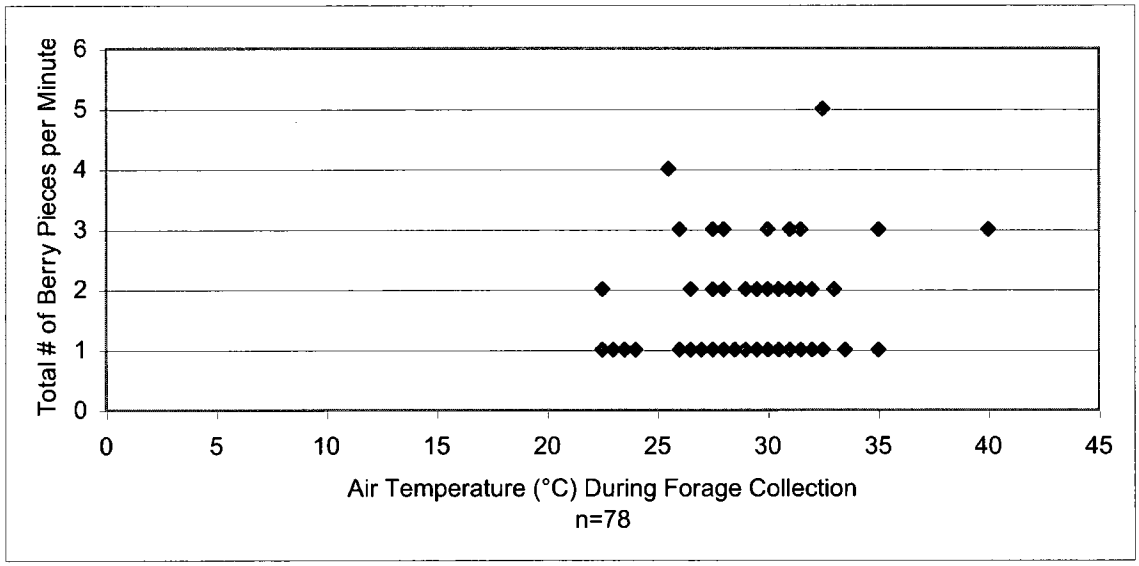

FIG. 50. Effect of air temperature on the numbers of berry plant pieces (*Vaccinium* sp. or *Gaylussacia baccata*) collected by nests during a one minute time period.

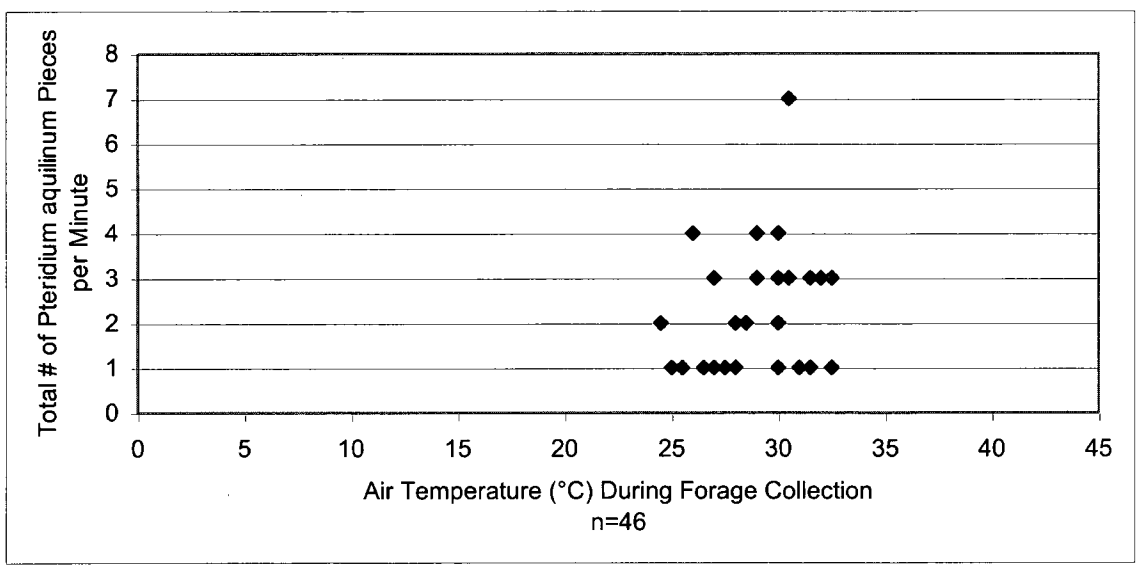

FIG. 51. Effect of air temperature on the numbers of *Pteridium aquilinum* pieces collected by nests during a one minute time period.

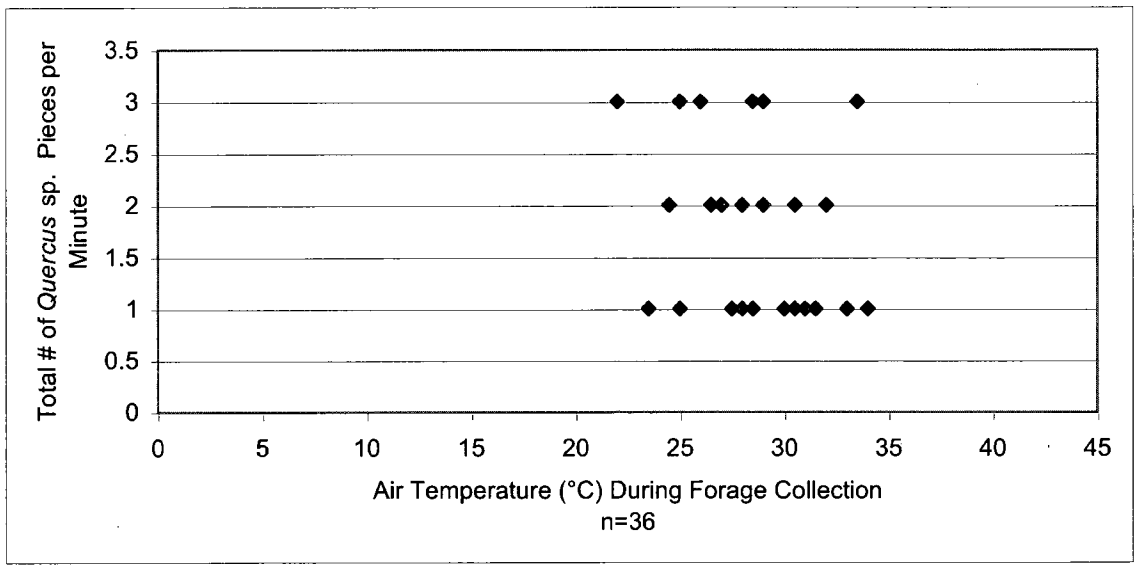

FIG. 52. Effect of air temperature on the numbers of *Quercus* sp. pieces collected by nests during a one minute time period.

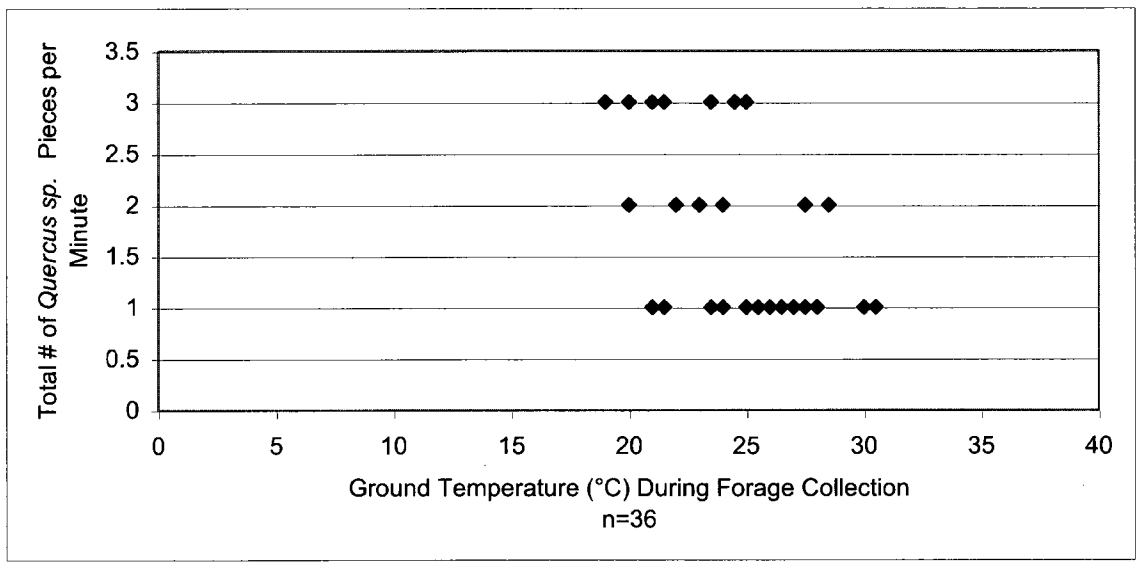

FIG. 53. Effect of ground temperature on the numbers of *Quercus* sp. pieces collected by nests during a one minute time period.

Figure 54 shows that the month of collection had a significant effect on the number pieces of forage collected during the study. Total forage fragments collected varied significantly between May (B=0.789; S.E.=0.225; Wald=12.273; df=1; p<0.001; Exp(B)=2.202), June (B=0.705; S.E.=0.197; Wald=12.768; df=1; p<0.001; Exp(B)=2.025), and July (B=0.430; S.E.=0.207; Wald=4.307; df=1; p=0.038; Exp(B)=1.538) and August and between August (B=-0.582; S.E.=0.216; Wald=7.225; df=1; p=0.007; Exp(B)=0.559) and September; collection of *Quercus* sp. (Figure 55) was significantly different between May (B=17.383; S.E.=0.775; Wald=503.498; df=1; p<0.001; Exp(B)=35000000), June (B=17.383; S.E.=0.665; Wald=683.885; df=1; p<0.001; Exp(B)=35000000), and July (B=16.549; S.E.=0.670; Wald=609.289; df=1; p<0.001; Exp(B)=15000000) and September. Other forage materials exhibiting significant difference between months (Figures 56 and 57) were berry parts (July-August: (B=1.417; S.E.=0.657; Wald=4.660; df=1; p=0.031; Exp(B)=4.127); August-September: (B=-1.680; S.E.=0.681; Wald=6.085; df=1; p=0.014; Exp(B)=0.186) and charcoal fragments (June-September: (B=16.559; S.E.=0.887; Wald=348.666; df=1; p<0.001; Exp(B)=16000000).

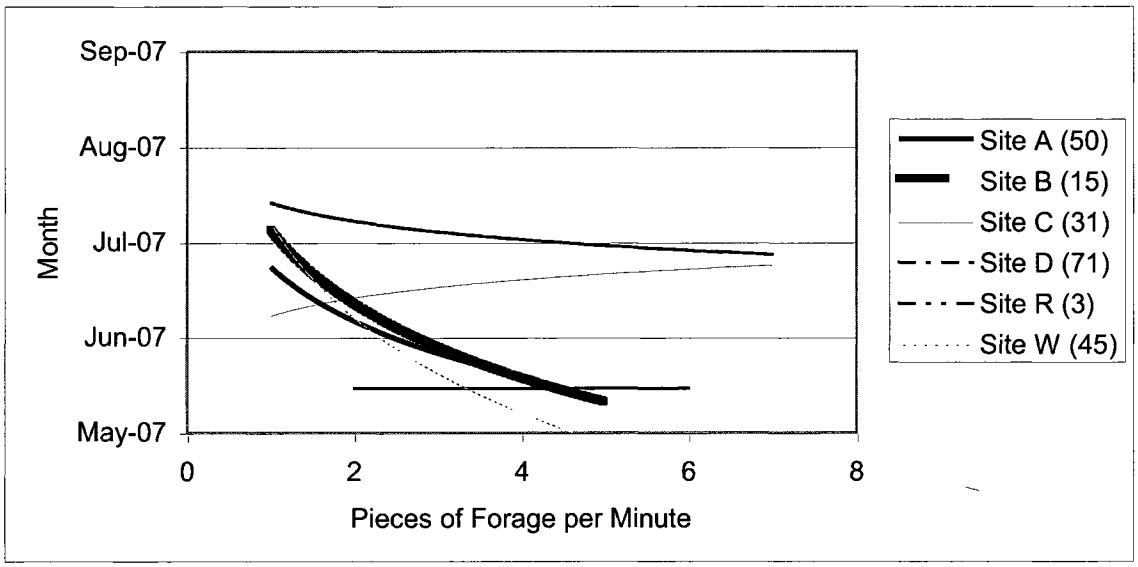

FIG. 54. Effect of month on the collection of general forage pieces per nest in different sites. Numbers in parenthesis (*) indicate sample sizes.

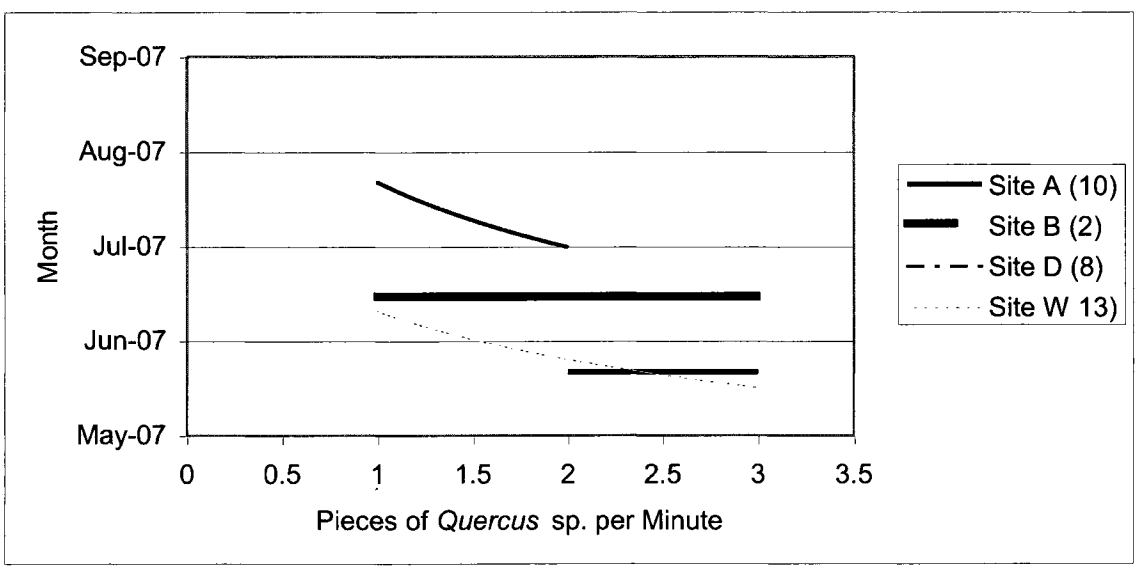

FIG. 55. Effect of population site on the collection of *Quercus* sp. leaves per nest in different sites. Numbers in parenthesis (*) indicate sample sizes.

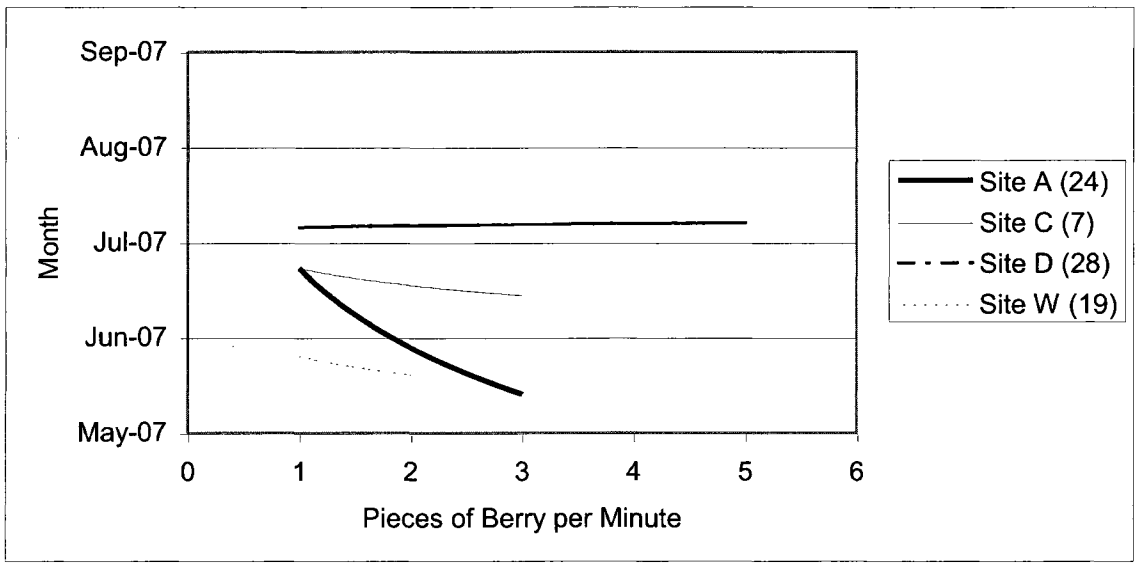

FIG. 56. Effect of population site on the collection of berry plant pieces (*Vaccinium* sp. or *Gaylussacia baccata*) per nest in different sites. Numbers in parenthesis (*) indicate sample sizes.

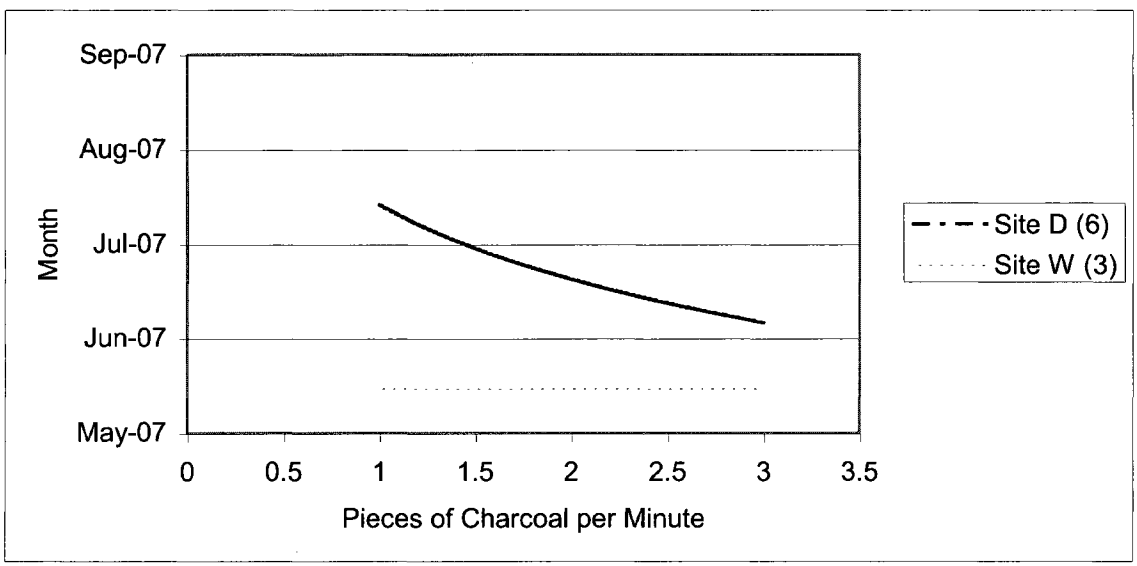

FIG. 57. Effect of population site on the collection of charcoal pieces per nest in different sites. Numbers in parenthesis (*) indicate sample sizes.

The number of fragments collected in different sites (Figures 54-59) varied significantly between *P. aquilinum* for site A and sites B (B=-2.107; S.E.=0.747; Wald=7.955; df=1; p=0.005; Exp(B)=0.122), C (B=-2.107; S.E.=0.747; Wald=7.955; df=1; p=0.005; Exp(B)=0.122), and D (B=-0.785; S.E.=0.389; Wald=4.086; df=1; p=0.043; Exp(B)=0.456), *Quercus* sp. for site A and sites C (B=19.545; S.E.=1.233; Wald=251.117; df=1; p<0.001; Exp(B)=310000000), D (B=3.354; S.E.=1.372; Wald=5.977; df=1; p=0.014; Exp(B)=28.627), and W (B=1.428; S.E.=0.648; Wald=4.850; df=1; p=0.028; Exp(B)=4.171), berry fragments for sites D (B=1.033; S.E.=0.451; Wald=5.254; df=1; p=0.022; Exp(B)=2.809) and W, and unknown materials for site R and sites A (B=-1.869; S.E.=0.771; Wald=5.876; df=1; p=0.015; Exp(B)=0.154), D (B=-0819; S.E.=0.353; Wald=5.370; df=1; p=0.020; Exp(B)=0.441), and W (B=-0.833; S.E.=0.394; Wald=4.469; df=1; p=0.035; Exp(B)=0.435).

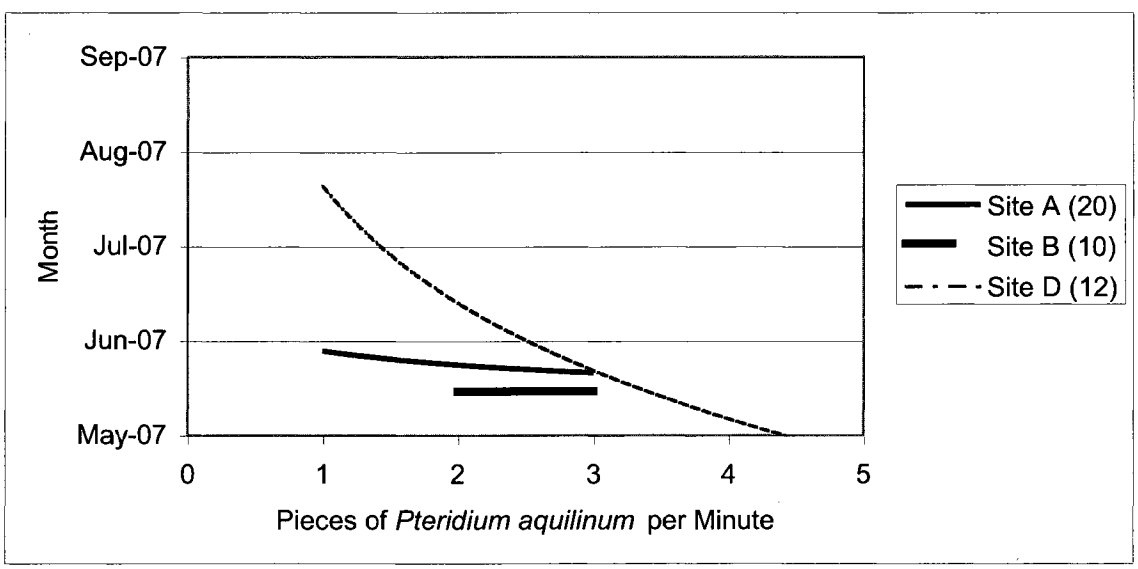

FIG. 58. Effect of month on the collection of *Pteridium aquilinum* pieces per nest in different sites. Numbers in parenthesis (*) indicate sample sizes.

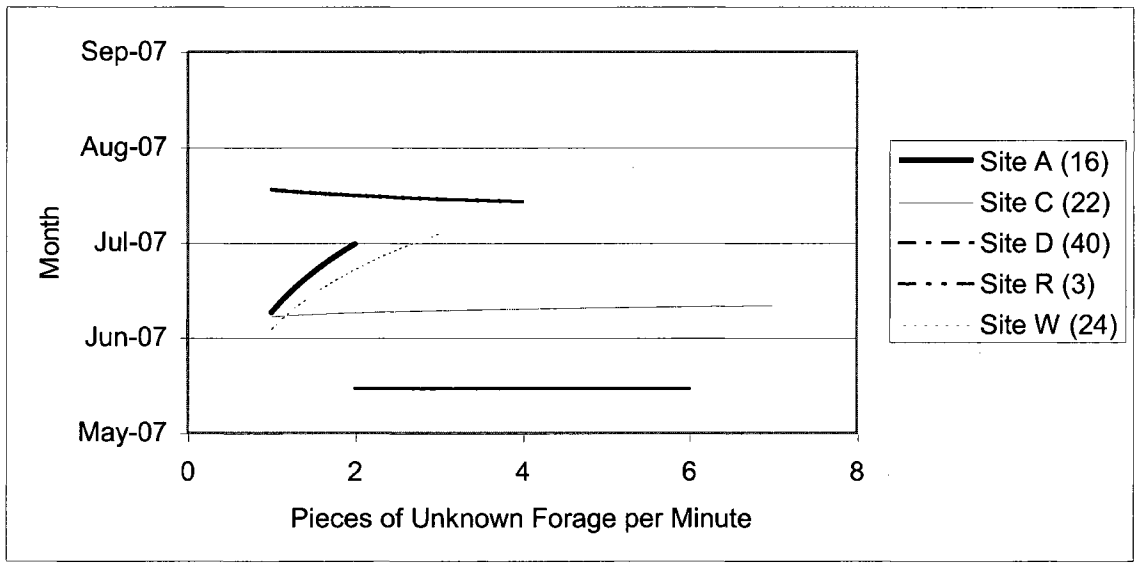

FIG. 59. Effect of population site on the collection of unknown material pieces per nest in different sites. Numbers in parenthesis (*) indicate sample sizes.

TABLE 14. Mean numbers of forage pieces collected per *T. septentrionalis* nest. Values in parenthesis (*) indicate the number of nests sampled.

Collection Date (2006)	Berry	*Pteridium aquilinum*	*Quercus* sp.	*Panicum* sp.	*Sassafras albidum*	*Pinus* sp.	*Opuntia compressa*	*Pyxidanthera barbulata*	Charcoal	Frass	Fungus	Unknown	Total
May 27	1.60 (5)	3.13 (8)	2.20 (5)	1.0 (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.77 (13)	2.88 (24)
June 4	2.0 (6)	2.08 (13)	2.50 (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.14 (7)	2.78 (23)
June 11	2.0 (6)	2.08 (13)	2.50 (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.25 (4)	2.70 (20)
June 25	1.39 (13)	0.0	1.0 (2)	1.0 (2)	0.0	2.0 (1)	0.0	0.0	2.0 (3)	0.0	0.0	1.13 (8)	2.60 (15)
July 9	1.0 (1)	0.0	2.25 (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.50 (2)	2.17 (6)
July 16	2.08 (13)	2.0 (3)	1.0 (2)	0.0	0.0	1.0 (1)	0.0	0.0	2.0 (5)	0.0	0.0	1.50 (10)	2.54 (24)
July 30	1.56 (9)	1.0 (1)	1.33 (3)	0.0	0.0	0.0	0.0	0.0	1.0 (5)	0.0	0.0	1.11 (9)	2.0 (17)
August 6	1.18 (11)	1.33 (3)	1.67 (3)	0.0	0.0	0	2.50 (2)	1.0 (1)	0.0	0.0	0.0	1.13 (8)	1.85 (20)
August 20	0.0	0.0	1.25 (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.30 (10)	2.33 (12)
August 27	1.33 (3)	1.0 (1)	1.50 (2)	0.0	2.0 (1)	0.0	0.0	0.0	0.0	1.0 (1)	0.0	1.73 (22)	1.75 (28)
September 3	2.0 (10)	1.25 (4)	1.0 (3)	0.0	0.0	0.0	0.0	1.0 (3)	1.0 (1)	1.0 (3)	5.0 (1)	1.29 (14)	2.64 (22)
September 16	3 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0 (1)	4.0 (1)
September 24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.67 (3)	0.0	0.0	1.67 (3)

A total of 515 pieces of forage material was collected from 215 nests, some of which may have been observed more than once in the season, in 2005. After drying, this forage material produced a total weight of 0.503 g, or 0.00112 g/piece of forage material. The initial weight of the total forage material was 1.3095 g, indicating that 61.6% of the sample weight was water mass.

Nest Contents

A total of four nests, one nest from each of the four sites in BEP, were excavated to determine composition of a *T. septentrionalis* nest in regards to numbers of workers, alates (female and male), and pupae and masses of fungal gardens and waste materials (Table 15). The mean number of workers captured per nest was 273.8 but ranged from 85 to 691 workers. The mean mass of a single worker was 0.53 mg. Female alates were found in two of the four nests. A mean of 32 female alates were found per nest, and they produced a mean mass of 1.15 mg per ant. Only nine male alates were found during the excavations resulting in a mean mass of 0.71 mg per ant. Pupae were found in two of the excavated nests. The two sets of pupae could be differentiated as workers or alates by their relative sizes (Figure 60). Alate pupae (1.03 mg per ant) were significantly larger ($F=9.307$; $p=0.016$) than the worker pupae (0.84 mg per ant). Fungal gardens and waste materials were collected from three nests with dry mean masses of 1.59 g and 2.16 g, respectively.

TABLE 15. Total numbers and mean masses of *T. septentrionalis* workers, alates, pupae, fungus gardens, and waste materials collected from four representative nests within BEP.

Study Site	Caste	Individuals Collected	Dry Mean Mass (mg)
A	Female Alate	8	0.89
	Worker	195	0.61
	Pupae (Alate)	48	1.03
	Garden Fungus	1	2985.90
	Waste Fungus	1	3197.90
B	Worker	85	0.38
	Waste Fungus	1	485.85
C	Female Alate	56	1.15
	Male Alate	9	0.71
	Worker	691	0.55
	Pupae (Worker)	34	0.84
	Garden Fungus	1	1386.50
D	Worker	124	0.40
	Garden Fungus	1	412.10
	Waste Fungus	1	2807.60

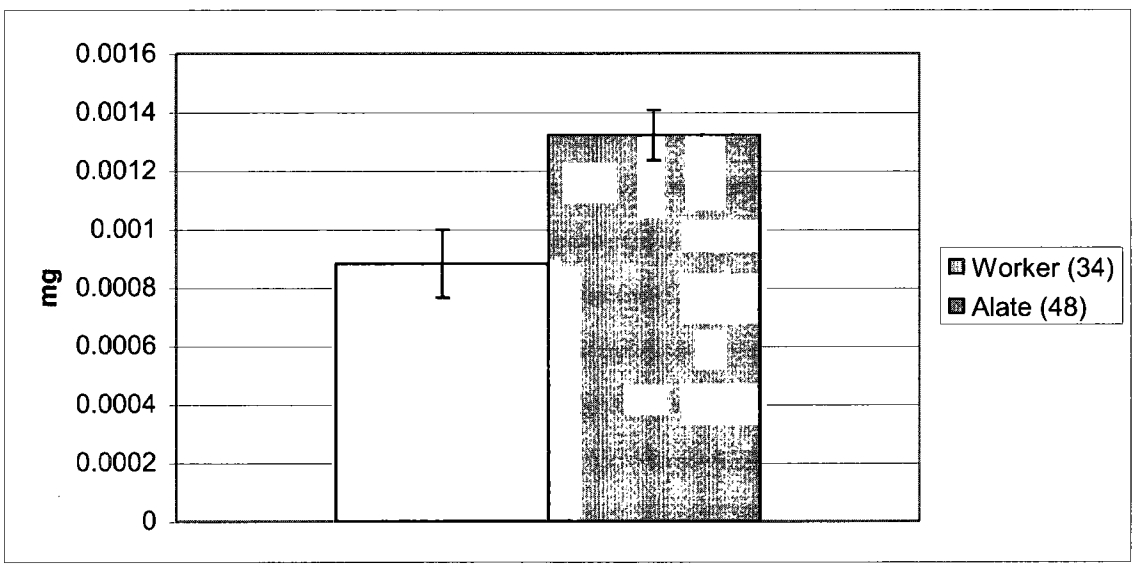

FIG. 60. Comparison of worker and alate pupae masses. Bars represent standard error. Numbers in parenthesis (*) indicate sample sizes.

Carbon/Nitrogen Composition

The highest concentrations of C (413.1 μg/mg) and N (94.1 μg/mg) were found in the adult worker members of the *T. septentrionalis* colony (Table 16). *T. septentrionalis* C concentrations were significantly higher than the fungal garden ($F=21.713$; $p<0.001$) and waste materials ($F=154.678$; $p<0.001$) (Figure 61). The only exception to this trend was the freshly harvested mass forage samples that demonstrated the highest concentration of C (436.2 μg/mg), and were significantly higher than the *T. septentrionalis* component ($F=8.815$; $p=0.008$), ant forage ($F=6.995$; $p=0.02$), fungal garden ($F=33.476$; $p<0.001$), and waste material ($F=268.06$; $p<0.001$). The ants' fungus garden (199.9 μg/mg) had a significantly lower C concentration ($F=13.15$; $p=0.005$) than the ant forage (282.9 μg/mg), but the fungal garden had a significantly higher C concentration ($F=10.543$; $p=0.007$) than waste materials (65.7 μg/mg).

TABLE 16. Carbon and nitrogen concentrations of each component of the *T. septentrionalis*/fungus garden symbiotic relationship. Numbers in parenthesis (*) indicate sample sizes.

Symbiont Component	C (μg/mg)	N (μg/mg)	C/N Ratio
T. septentrionalis (13)	367.6	87.1	4.24
Pupae (4)	354.2	83.9	4.27
Worker (2)	331.0	74.1	4.49
Alate (2)	377.3	93.6	4.05
Female Alate (3)	355.5	79.8	4.42
Male Alate (2)	321.8	90.3	3.57
Adult Worker (4)	413.1	94.1	4.39
Ant Forage (7)	282.9	19.0	23.31
Mass Forage (8)	436.2	20.8	30.92
Fungal Garden (7)	199.9	16.9	12.33
Waste (6)	65.7	4.2	16.56

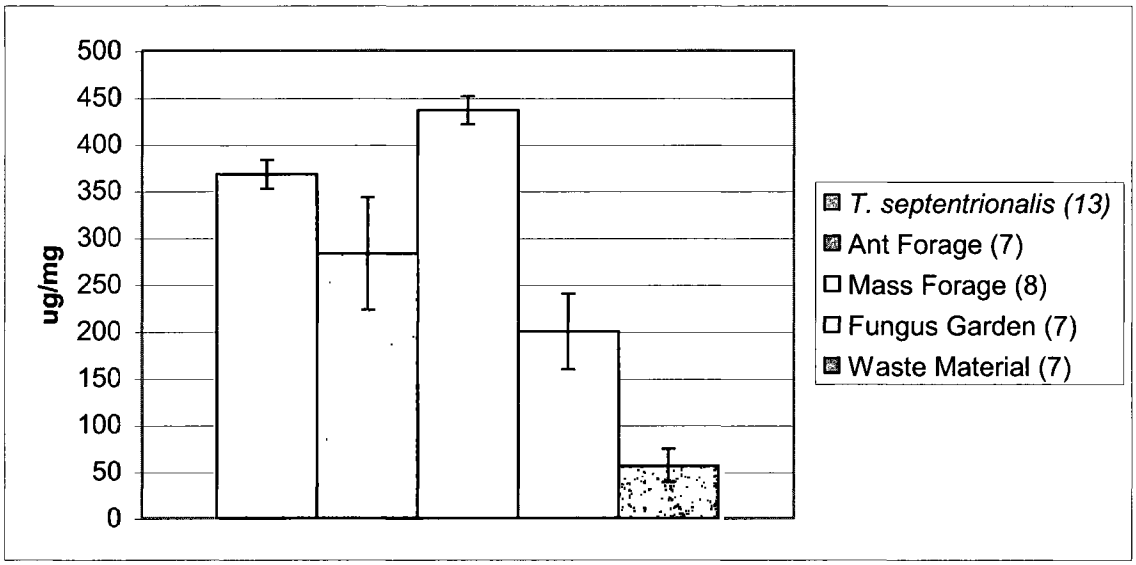

FIG. 61. C concentrations of multiple components of the *T. septentrionalis*/fungal garden symbiont. Bars represent standard error. Numbers in parenthesis (*) indicate sample sizes.

N concentrations lowest in the ant component, worker pupae = 74.1 μg/mg, but this was over 3.5 times higher than any other non-ant component (Table 16). All other symbiont components had significantly lower N concentration than *T. septentrionalis*: ant forage ($F=114.355$; $p<0.001$), mass forage ($F=174.66$; $p<0.001$), fungal garden ($F=232.27$; $p<0.001$), and waste materials ($F=446.676$; $p<0.001$) (Figure 62). Waste materials had significantly lower N concentrations than *T. septentrionalis* ($F=446.676$; $p<0.001$), ant forage materials ($F=13.15$; $p=0.004$), and mass forage materials ($F=268.06$; $p<0.001$).

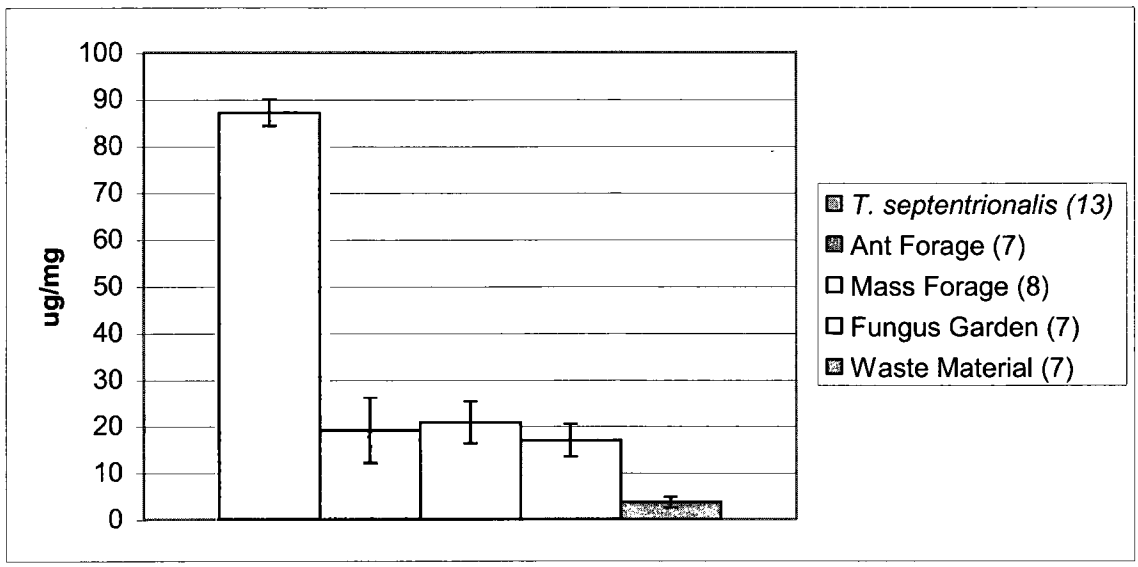

FIG. 62. N concentrations of multiple components of the *T. septentrionalis*/fungal garden symbiont. Bars represent standard error. Numbers in parenthesis (*) indicate sample sizes.

The mean C/N ratio of forage (23.31 and 30.92) and waste materials (16.56) were found to be approximately 3.5 times higher than that of the *T. septentrionalis* colony (4.24), while the fungal garden C/N ratio (12.33) was over 2.5 times higher than that of the *T. septentrionalis* colony (Table 16). These results produce significant differences between the *T. septentrionalis* component and all other forage and fungal components: ant forage ($F=8.047$; $p=0.011$), mass forage ($F=21.520$; $p<0.001$), fungus garden ($F=223.687$; $p<0.001$), and waste materials ($F=18.992$; $p<0.001$) (Figure 63). The mass forage samples also had a significantly higher C/N ratio waste materials ($F=5.359$; $p=0.038$) than the fungal garden.

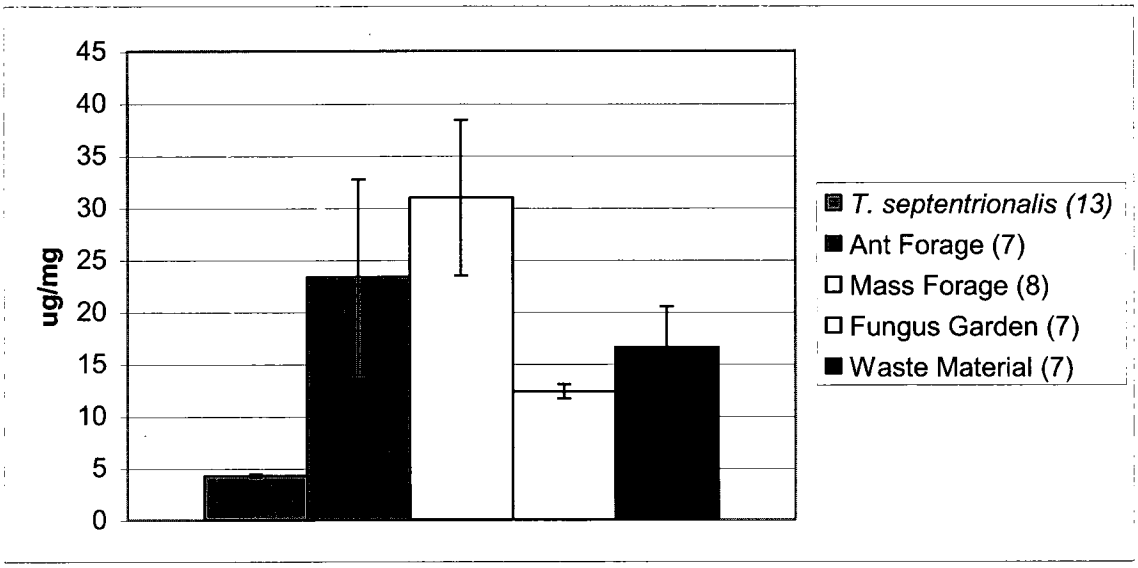

FIG. 63. C/N ratios of multiple components of the *T. septentrionalis*/fungal garden symbiont. Bars represent standard error. Numbers in parenthesis (*) indicate sample sizes.

Macronutrient and Micronutrient Composition

Sample masses were too small for replicates to be analyzed, therefore statistical analysis were not performed. The macronutrient concentrations in fungal sample were higher than those found in the mass forage samples for 5 of the six macronutrients, and the concentration of potassium in the forage samples was less than 5% higher than the fungus sample (Table 18). *T. septentrionalis* had greater than 60% increase in concentration than the fungal sample for phosphorous, sodium, and sulfur, but the fungus had greater than 60% increase in potassium concentration than the T. septentrionalis sample. Waste material had lower concentrations for all of the macronutrients than the fungal garden and the ants.

Micronutrient and heavy metal results were similar to those of the macronutrients in most respects (Table 19). The fungal garden was higher the forage samples for all of the micronutrients and metals. *T. septentrionalis* had higher concentrations of all of the

micronutrients and metals than the fungal garden with the exceptions of boron and manganese. Unlike the macronutients, the waste material contained higher concentration than the fungus, ants, or both for many of the micronutrients and metals: aluminum (both), boron (ants), iron (both), lead (both), and manganese (both).

TABLE 17. Macronutrient concentrations of each component of the *T. septentrionalis*/fungus garden symbiotic relationship. Numbers in parenthesis (*) indicate sample sizes.

Symbiont Component	Ca (μg/g)	Mg (μg/g)	P (μg/g)	K (μg/g)	Na (μg/g)	S (μg/g)
T. septentrionalis (1)	766.62	312.02	780.74	956.26	1119.81	570.04
Mass Forage (3)	427.75	253.14	349.84	1621.35	57.98	203.54
Fungal Garden (1)	678.3	361.18	488.6	1545.62	161.28	292.4
Waste (1)	410.65	183.23	218.25	779.27	58.07	153.78

TABLE 18. Micronutrient and heavy metal concentrations of each component of the *T. septentrionalis*/fungus garden symbiotic relationship. Numbers in parenthesis (*) indicate sample sizes.

Symbiont Component	Al (μg/g)	B (μg/g)	Cd (μg/g)	Cu (μg/g)	Fe (μg/g)	Pb (μg/g)	Mn (μg/g)	Ni (μg/g)	Zn (μg/g)
T. septentrionalis (1)	434.27	14.64	3.39	59.64	488.75	6.62	332.93	18.14	661.13
Mass Forage (3)	40.47	15.6	N/A	12.02	50.42	1.61	166.03	2.98	29.97
Fungal Garden (1)	369.82	21.53	N/A	16.13	228.29	6.37	601.53	4.37	54.25
Waste (1)	1156.72	16.23	N/A	9.98	848.48	8.88	549.53	3.06	30.30

Caloric Content

Due to limited sample sizes, replicates were only possible for the mass vegetation, fungal garden, and nest waste samples (Table 16). The fungal garden samples were all higher than the next highest sample from any of the other components. The caloric content of the *T. septentrionalis* sample was higher than the actual forage collected from the ants and the waste deposited outside the nests. Mass forage sample results were similar to those found with the C/N composition (see above) to the extent that they were higher than the value from the forage materials collected directly from the ants.

TABLE 19. Caloric content of each component of the *T. septentrionalis*/fungus garden symbiotic relationship. Numbers in parenthesis (*) indicate sample sizes.

Symbiont Component	Caloric Content (kcal./g)
T. septentrionalis (1)	5.44
Ant Forage (1)	4.07
Mass Forage (3)	4.5
Fungal Garden (2)	3.47
Waste (2)	2.7

CHAPTER IV

DISCUSSION

ABIOTIC CONDITIONS

In previous studies of tropical attine species (*A. cephalotes* and *A. sexdens*), activity patterns of colonies have been described as random and variable (Beebe 1921, Lutz 1929, Weber 1941, Eidmann 1935, Hodgson 1955). The first portion of this research was designed to establish the abiotic conditions that influence the activity of *T. septentrionalis* colonies within Blackwater Ecologic Preserve.

Temperature

Lewis *et al.* (1974a-b) found that temperatures ranging from 21.5 to 27.5°C had no significant effect on the foraging activities of *A. cephalotes* in Trinidad. This temperature range is above the mean temperature of activity for the colonies of *T. septentrionalis* observed in this study. However, active temperature ranges reported for attine species inhabiting higher latitudes are far more similar to those collected in BEP. In Louisiana, recorded temperatures for activity of *A. texana* colonies range from 11 to 29°C (Moser 1967), and *T. smithi* have been shown to be active between 16-38°C (Schumacher and Whitford 1974). Weber (1956a) established minimum temperatures for *T. septentrionalis* activity in Florida and New Jersey at 13-15°C, with sluggish behavior beginning at 18°C. *T. septentrionalis* activity was limited to the months of April through October in New Jersey, but Florida colonies were able to function throughout the year (Weber 1956a). The temperatures and dates recorded in the current study are more similar to those of the New Jersey colonies than the Florida colonies.

T. septentrionalis occupy a diverse climatic and geographic range, but their habitat within that range is restricted. Temperature regimes within acceptable habitats determine the activity patterns of *T. septentrionalis* on a daily and annual scale. At the northern limit of the *T. septentrionalis* range, activity is limited to the warmer months of the year (April-October), but during these months, the ants experience daily extremes. Early spring and late fall produce ant activity during the middle of the day when temperatures are at their highest; midsummer produces activities in the morning and late afternoon before and after temperatures have peaked. Management of their activity reduces temperature, water, and energy stress on individual ants.

Norberg (1977) has hypothesized that seasonality of a species is a consequence of limited resource availability (vegetation), which is primarily controlled by temperature. As the costs associated with resource procurement increase, the benefits gained from the activity are so severely reduced that all activity is eventually halted.

Light and Humidity

Hodgson (1955) stated that activity of colonies of *A. cephalotes* was principally controlled by light intensities of three lumen and a latent physiological rhythm. Ant activities were not observed until light levels reached three lumen in the early morning hours, and activities ceased shortly after levels were reduced below three lumen in the evenings. Lewis et al. (1974a-b) defined a range of ant activity associated with light intensity of 0.5 to 100000 lumen, leading to the conclusion that photoperiod had no effect on ant activity. These findings were further supported by Cherrett (1968) who observed both nocturnal and diurnal activity in *A. cephalotes* in Panama. *T. septentrionalis* activity in Louisiana was observed only during late afternoons, evenings,

and early mornings (Cole 1939), which may have been more closely associated with temperature extremes than light intensity.

Soil Conditions

Only Cole (1939) has produced a detailed citation of the condition of the soils in which *T. septrentrionalis* excavates nests. Those findings indicate that *T. septrentrionalis* make their nests in soils with dry, sandy upper layers (0-16.5 cm) which transition to wet, sandy soils. The current study demonstrated that *T. septrentrionalis* predominantly occupy soils with lower moisture levels in the range of 30-60 cm since the colonized areas did not differ significantly in soil moisture from the uncolonized areas from 0-30 cm and 60-90 cm. These findings also appear to directly influence the ant and plant community structure as discussed below.

The location of *T. septentrionalis* populations and the ant and plant community structure surrounding these populations are principally governed by abiotic conditions. *T. septentrionalis* occupy sandy soils, which are widely available, but the amount of water in the soils determines the suitability of the site. Water levels are related to the density of the soil, which influences the ability of the ants to excavate, and also influences the growing conditions of the fungal garden. In addition to the direct impact on *T. septentrionalis* colonies, the amount of available water in the soil also determines the diversity and density of local vegetation. Drier soils retard the growth of vegetation and produce open areas that are more easily occupied by *T. septentrionalis*.

BIOTIC CONDITIONS

Nest Distribution

Colonies of *T. septentrionalis* within BEP occurred in relatively sparse aggregates of 0.072 to 0.145 nests per m^2, compared to colonies in Florida occurring in densities of 0.19 to 0.3 nests per m^2 (Lenczewski 1985). Lenczewski explained the colony densities by intraspecific competition. The Fretwell-Lucas model (Fretwell and Lucas 1970) says that coarse grained species should reduce densities as resource quality and quantity decrease. Wiens (1976) also attributes colony densities, and population distribution, to habitat quality. Species with well-defined environmental requirements will exhibit tendencies toward patch selection, while generalists will be more evenly distributed. Decreasing habitat quality or low population numbers have also been linked to the aggregation of species (Wiens 1976). The *T. septentrionalis* colony aggregates were located 50 m or more from one another in BEP. These findings, when combined with the abiotic conditions discussed above, agree with Theunis *et al.* (2005) that distribution of a species at scales above 50 m are controlled by environmental conditions.

Ant Community Structure

At BEP the ant community survey found that areas populated by *T. septentrionalis* have fewer, although not significantly, species of ants (27 versus 30), less diversity of ant species (2.47 versus 2.61), and less evenness (0.75 versus 0.77) than unpopulated areas. Increased disturbance produces lower species richness and evenness and increased dominance by fewer species (Roth *et al.* 1994). There were three ant species (*A. interjectus*, *L. flavus*, and *N. texanus*) that were found in the *T.*

septentrionalis populated areas that were not found in the unpopulated areas. *A. interjectus* and *L. flavus* both share the nesting preference of subsoil nests in open areas with access to ample sunlight (Brian 1977). *N. texanus* also shows a preference for ground nesting but has also been found in decaying logs and stumps.

Areas without *T. septentrionalis* contained seven species (*D. bureni*, *F. subsericea*, *L. alienus*, *P. clypeata*, *P. faisonensis*, *P. pennsylvanica*, and *P. vividula*) that were not found in populated areas. As with the species that were found in populated areas, the common denominator among these species was nesting site preference. *D. bureni* (Trager 1988), *P. faisonensis* (Trager 1984), and *P. vividula* (Trager 1984) are found most frequently in highly disturbed areas with moist soil conditions. *F. subsericea* (Say 1836), *L. alienus* (Wilson 1955), and *P. pennsylvanica* (Buckley 1866) each demonstrate preferences for damp sites such as decaying logs and leaf litter.

Bestelmeyer and Wiens (1996) noted that attines are more abundant in higher disturbance areas. While *T. septentrionalis* is the only attine species studied in this research, this species was also only identified in areas exhibiting regular disturbance. These findings are supported by the presence of other ant species in areas inhabited by *T. septentrionalis* that are closely associated with disturbed, open areas.

Vegetation Analysis

As with the ant community, areas populated by *T. septentrionalis* had fewer species of plant (15 versus 22), less species diversity (1.37 versus 2.13), and less species evenness (0.51 versus 0.69) than control areas that were uninhabited by *T. septentrionalis*. Only one plant species, *S. erecta*, was unique to the *T. septentrionalis* populated sites. A defining characteristic of *S. erecta* is dry, open soils (Flora of North

America vol. 20, pp. 121). Two species (*O. compressa* and *S. albidum*) not found in plant sampling in the *T. septentrionalis* populated sites, but each was recorded as being actively foraged by *T. septentrionalis*. Both of these species occupy disturbed habitats, and *O. compressa* prefers well drained soils (Flora of North America vol. 3, pp. 30; Benson 1962). Five species of plants (*C. rosea*, *L. benzoin*, *L. styraciflua*, *P. polygama*, and *S. perichaetiale*) were found only in areas not occupied *T. septentrionalis*. Each of these species is strongly associated with wet habitats (Flora of North America vol. 3, pp. 28, 367; vol. 5, pp. 538; vol. 23, pp. 289; vol. 27, pp. 54).

Foraging Analysis

Atta are diverse in their selection of forage materials. Cherrett (1968) found that *A. sexdens* sampled 50% of the vegetation species surrounding their nests. *A. colombica* foraged from 49-77% of the vegetative species surrounding their nest (Rockwood 1975, 1976). Despite this wide range of foraged materials, *Atta* species focus their foraging activities around a smaller range of available vegetative species. Only 22% (13 of 59) of foraged plants were preferred by *A. cephalotes* based on the amount of materials returned to the nests. *A. colombica* preferred 31.4% (27 of 86) of the overall species that they foraged from (Rockwood 1975, 1976). Six species of plants represented 97% of the foraged materials for *A. cephalotes* (Blanton and Ewel 1985).

Lower attines also demonstrate foraging preferences. *T. turrifex* foraged 69.6% plant parts and 17.4% caterpillar frass (Waller 1989) and Lenczewski (1985) found that *T. septentrionalis* focuses primarily on oak leaves, flowers, plant debris, and caterpillar frass. The colonies of *T. septentrionalis* in the current study were found to forage from

53.3% (8 of 15) of the available plant species, but they focused their foraging on the berry, oak, and bracken fern plant materials.

While forage selection activities appear similar among the various species of attines, foraging masses vary greatly between tropical and temperate species. Weber (1966) calculated a colony of *A. sexdens* foraging 5892 kg of plant material over a 77 month period. This would produce substrate intake of approximately 2.55 kg/day. If a *T. septentrionalis* nest in BEP was assumed to forage for a continuous 24 hour period, then the estimated intake for that day, using the mean foraging rate, would be 10.06 g. The low and high foraging rates estimate a daily intake of 4.2-29.4 g. While these numbers indicate an obvious disparity between higher and lower attines, large differences can also be found in foraging rates between populations of *T. septentrionalis* occurring in different regions of the United States. Colonies of *T. septentrionalis* in Florida forage at a maximum rate of 0.77 pieces of forage per minute, with a mean rate of 0.24 pieces/minute (Lenczewski 1985). These calculated rates are much lower than those observed in Virginia, where the mean rate of foraging was 2.4 pieces/minute.

The water content of forage materials affects selectivity. Bowers and Porter (1981) found that *Atta* prefers vegetation with a high water content (up to 80%), and palatability can be correlated with water content (Cherrett 1968, 1972, Rockwood 1972, 1976). This increase inpalatability may be due to a dilution of phyochemicals within the plant tissues (Mattson 1980). Feeny (1970) determined that young oak leaves are a preferred forage material because of their higher water content and lower tannin concentration. Oak leaves were one of the four major groups of preferred substrate for the *T. septentrionalis* colonies observed in the current research and in Florida

(Lenczewski 1985). A further aspect of Bowers and Porter's (1981) research indicated that this preference was also affected by distance from the nests. Ants foraging within a range of 20 m preferred vegetation with a water content of 65%; as foraging distances neared 90 m, water content of foraged materials escalated to 80%. This preference for higher water content as foraging distances from the nest increase may be a mechanism used to offset the effects of desiccation encountered during foraging (Holling 1959). Foraging *T. septentrionalis* in BEP were never observed to forage greater than 5 m from a nest, and the findings of a 61.6% water content are in agreement with Bowers and Porter (1981).

Nest Contents

The current study found colonies of *T. septentrionalis* in BEP to number a mean of 273.8 workers per colony. This number agrees with the previous studies of Weber (1972), Lenczewski (1985), and Beshers (1993) that found colony means ranging from 80 to 800 workers per nests. Beshers (1993) correlated worker numbers with the ability of colonies to effectively produce alates, stating 400 workers would represent a threshold below which alate production would be limited. The finding in the present study of 8 mature alates in a colony of 195 workers when compared to 65 alates in a colony of 691 workers appears to support this claim.

Worker sizes within *T. septentrionalis* colonies have been hypothesized to indicate the quality of surrounding habitat (Lenczewski 1985). The mean worker size of *T. septentrionalis* in 34 colonies from Florida was 0.52 mg, which is the same as the mean worker size in 4 colonies excavated in BEP (0.53 mg). However, the range of the Florida workers (0.45 to 0.75 mg) is much greater than the workers of BEP (0.38 to 0.61

mg). These differences in range may be indicative of a numerical anomaly produced by the differing sample sizes, a shorter foraging season, or a decreasing quality of the habitat encountered by the northern populations of *T. septentrionalis*.

The small colony and worker size and transient nature (Lenczewski 1985) of the *T. septentrionalis* colonies, which survive an average of 1.7 years, are indicative of a species populating an energy poor habitat. By producing colonies of with fewer workers, *T. septentrionalis* is able to mature more rapidly and begin the reproductive process at an earlier stage of colony development. The small sizes of the workers and alates allow limited resources to be partitioned for their development, while producing higher numbers and increasing the likelihood of the establishment of new, successful colonies.

Carbon/Nitrogen Composition

Attines consume the fungal gardens of their nests as their primary source of nutrition (Weber 1972, Holldobler and Wilson 1990, Silva *et al.* 2003). These fungal gardens are grown on a substrate supplied by the foraging activities of the ants. The fungal gardens of *Acromyrmex* sp. and *Atta* sp. are capable of digesting many polysaccharides (Richard *et al.* 2005 and Bacci *et al.* 1995) includes cellulose as a C source for attine fungal gardens, but the availability of cellulose to the fungal gardens is refuted by (Abril and Bucher 2004). Abril and Bucher (2004) also found that only soluble N sources were readily available to the fungal gardens. These findings are significant because the inability of the fungal gardens to breakdown insoluble C and N compounds increases the workload placed on the ant colonies to insure that enough C

and N are made available to their fungal gardens to flourish and supply with nutrition to the colony.

Bucher *et al.* (2004) analyzed the change in C and N between the forage and waste materials of *Acromyrmex lundi*. Total and soluble C were found to decrease between forage and waste compartments, but total and soluble N increased. These results produced a minor decline in the C/N ratio, which is counter to the results observed in the current study. High C/N ratios for forage materials of *A. cephalotes* have also been observed by Howard (1987), while low C/N ratios have been found for the waste materials of *A. colombica* (Hudson 2005). These differences in results appear to be produced by the types of forage gathered by the respective ant species (*A. lundi* collect primarily fresh cut leaf materials, while *T. septentrionalis* collects primarily detrital materials) because when mass forage samples from the current study are compared to waste materials, results are similar to those of Howard (1987), Bucher *et al.* (2004), and Hudson (2005). *T. septentrionalis* forage less nutritional substrate than their tropical counterparts, but their fungus gardens appear to be more efficient in the removal of nutrients. This may indicate the fungal gardens of *T. septentrionalis* are capable of digesting cellulose and thereby reduce the foraging stresses that would be placed on the colonies otherwise.

In addition to required resources being limited by member composition and individual size within a colony, efficient selection and use of fungal substrate optimizes available resources. *T. septentrionalis* forage primarily on detrital materials, and their fungal gardens extract over 70% of the C and N provided by the substrate. The fungal gardens may also play a limited role in providing accessible nutrients to the surrounding

soils in the form of yearly waste heaps deposited outside of the colonies by the ants with high C/N ratios.

Macronutrient and Micronutrient Composition

In equilavent studies performed on *A. sexdens* in Brazil (Moutinho *et al.* 2003) and *A. colombica* in Panama (Haines 1978, Hudson 2005), nutrient concentrations in the forage and refuse generally greatly exceeded those of the current study, sometimes by as much as 51 times. The potassium concentration in the refuse was one of the few exceptions, where the current study found a higher concentration than Moutinho *et al.* (2003): 779.27 μg/g versus 602.6 μg/g. Comparison with the results from Haines (1978) resulted in two higher concentrations for the current study: forage copper 12.02 μg/g versus 8.4 μg/g and waste manganese 549.53 μg/g versus 271.9 μg/g.

Caloric Content

The caloric values derived from the current study are similar to those gathered in analysis of other species of foraging ants. Seal and Tschinkel (2008) evaluated the caloric content of *T. septentrionalis* to be between 4.19 kcal/g and 5.44 kcal/g depending on the nutrient availability within each of their experimental treatments. An alternative method for calculating the caloric value of *T. septentrionalis* is to use the standard ant value of 2.5 kcal/g live weight, measured by Golley and Gentry (1964) for *Pogonomyrmex badius*, and adjusting for the water content of *T. septentrionalis*. The resulting value is 5.81. None of these values are greatly removed from the experimentally derived value of 5.44 kcal/g of this study. Energy from the forage materials of *A. colombica* was determined to be 4.25 kcal/g (Lugo *et al.* 1973) and 4.77 kcal/g (Haines 1978), but the forage collected directly from *T. septentrionalis* is 4.07

kcal/g while whole leaf samples of foraged plants produced a value of 4.5 kcal/g. No caloric values for fungal gardens could be located, but a mean value for edible fungi was calculated as 3.47 kcal/g, which is much higher than the 5.78 kcal/g attained in the laboratory for the fungal garden of *T. septentrionalis*. Lugo *et al.* (1973) also measured the calories in waste materials to be 2.5 kcal/g, which is similar to the derived value of 2.7 kcal/g of *T. septentrionalis* waste.

Since *T. septentrionalis* foraging was only observed in the field for 2-3 consecutive hours, data from prior studies of attines will be used to calculate the energetics of the *T. septentrionalis* symbiotic relationship. Measured foraging lengths of *A. cephalotes* in Panama ranged from 342-765 minutes in a day (Lewis *et al.* 1973). Using these forage period values and the measured rates of foraging (minimum = 1 piece of forage/minute, mean = 2.4 pieces of forage /min, and maximum = 7 pieces of forage /min) for *T. septentrionalis*, substrate foraging was calculated to be a minimum of 0.038 $g/m^2/d$, a mean of 0.091 $g/m^2/d$, and a maximum of 0.27 $g/m^2/d$ over a 342 minute time span per nest. Over 765 minutes, calculated forage intake was 0.085 $g/m^2/d$, 0.20 $g/m^2/d$, and 0.59 $g/m^2/d$, respectively. With the exception of the maximum intake values for both foraging lengths, all of the calculated intake rates are much lower than those measured for *A. cephalotes* in Costa Rica, which ranged from 3.47 $g/m^2/d$ to 0.27 $g/m^2/d$ (Blanton and Ewel 1985).

These forage rates can be converted to caloric rates using the values of 4.07 kacl/g and 4.25 kcal/g, for *T. septentrionalis* and *A. cephalotes*, respectively. Caloric intake for *T. septentrionalis* a nest of over the shortest foraging period would range from 0.15 to 1.08 $kcal/m^2/d$, while the longer foraging period produces a range of 0.35-2.42

kcal/m^2/d. The daily caloric intake of a nest of *A. cephalotes* ranges from 1.14 kcal/m^2/d to 14.75 kcal/m^2/d.

When direct comparisons are made between tropical attine and *T. septentrionalis* energetics, *T. septentrionalis* appear to extremely limited in the resources that are available to them. But while *T. septentrionalis* are limited by the length of the foraging season, the colonies are often 100 times smaller than tropical attine colonies. In this respect, a foraging differential of up to 92 times is not unexpected and may actually indicate that *T. septentrionalis* are more efficient in their utilization of the environment than their tropical cousins. Seal and Tschinkel (2008) described *T. septentrionalis* as living in suboptimal conditional because they do not fully exploit the resources that are made available to them.

A. colombica, and their fungal gardens, in Panama have been estimated to assimilate between 22% (Haines 1978) and 89% (Lapin *et al.* 2004) of the energy that is brought into the nest by foraging, but the latter value was deemed as unreasonable. Using the same foraging times as above to calculate a yearly input of calories into an individual *T. septentrionalis* nest, values range from a low of 29.58 kcal/m^2/yr (191 days of foraging for 342 minutes at 1 piece of forage per minute) to 462.17 kcal/m^2/yr (191 days of foraging for 765 minutes at 7 pieces of forage per minute); a foraging rate of 2.4 pieces/minute resulted in yearly intakes of 70.70 kcal/m^2/yr and 158.15 kcal/m^2/yr at 342 minutes and 765 minutes, respectively. Most of these values, except for the lowest, cannot be considered as valid due to the yearly intake only amounting to 46.6 kcal/m^2/yr (Haines 1978); however, Lugo *et al.* (1973) measured caloric intake at 126 kcal/m^2/yr. One possible explanation for this disparity in calculated intake rates may be a difference

in determining the surface area occupied by an individual nest (Lugo *et al.* 1973, Haines 1978), Blanton and Ewel 1985). The methods of the current study are more restrictive than those used in the tropical studies partially because a larger number of nests are used to establish the nest areas of *T. septentrionalis* than the various *Atta* sp. Blanton and Ewel (1985) used the total area of forage in their calculations of intake, but the actual surface area of their study nests was only 6.75% of that total. This indicates that *Atta* sp. are sampling from a larger physical foraging area than *T. septentrionalis*.

With the above concerns taken into account, only the caloric intake value of 29.58 kcal/m^2/yr for *T. septentrionalis* nests will be considered further. At a 22% assimilation rate, a nest (ants and fungal garden) can be expected to retain 6.51 kcal/m^2/yr or approximately 66% of the intake recorded for *A. colombica* (Haines 1978). Further evidence for the unlikelihood that these values would be so close to one another can be found in the difference in energy expenditure required for foraging substrate. The leaf cutting behavior of *A. sexdens* requires 31 times more energy than resting (Roces *et al.* 1997), but *T. septentrionalis* acquire most of their substrate by foraging detrital materials.

While the calculated values may be unexpectedly low for other attines, the yearly caloric intake of 6.51 kcal/m^2 for *T. septentrionalis* can be supported by their life history. The intake rate of 6.51 kcal/m^2/yr is based on 100% commitment by the workers, but only 16.89% of the labor performed in a *T. septentrionalis* nest involves foraging (Beshers 1993), which reduces the intake the rate of individual nests to 1.1 kcal/m^2/yr or 11.1 kcal/nest/yr based on a nest density of 0.1 nest/m^2. Since *T. septentrionalis* colonies reach maturity within the first year of settlement and survive only 1.7 years

(Lenczewski 1985), nest contents can be assumed to be the biomass from one year of production. In the current study, the mean *T. septentrionalis* nest contained 0.27 g ants and 1.59 g of fungus. When the nest contents are converted to calories, the result is a total of 6.96 kcal/nest/yr. This value is 62.7% of the intake rate based on assimilation calculations; however, this intake value based on biomass does not take into account refuse. The mean refuse pile of a *T. septentrionalis* nest is 2.16 g and provides an additional 5.83 kcal/nest/yr to the biomass calculation. The addition of refuse brings the total biomass calories to 12.79 kcal/nest/yr, 15.2% more than the 11.1 kcal/nest/yr derived by applying the assimilation rate.

The refuse produced by *T. septentrionalis* is calorie poor, but that refuse is concentrated into a smaller area than the detrital matter of the forest floor. These refuse piles provide a calorie source of 5.83 kcal that is localized in a defined space. Since the fungus garden does not remove all of the nutrients from the substrate, and initializes the breakdown of complex nutrients, these refuse piles represent small, but fertile, islands in the environment (Bucher *et al.* 2004, Hudson 2005).

CHAPTER V

CONCLUSION

1) Mechanisms determining population densities: Site-specific physical factors (cover density, light intensity, soil composition) have a greater influence on population densities than interspecific competition. This hypothesis was tested by comparing plant and ant species counts and densities within *T. septentrionalis* populated and unpopulated areas.

T. septentrionalis remain active in the environment across a wide spectrum of above ground abiotic conditions. Light intensity and soil and air temperatures play significant roles in the activity patterns of *T. septentrionalis* colonies in BEP. Low temperatures are most associated with limiting levels of activity during the early spring and late fall seasons, while high temperatures reduce activity during the summer season. Both of these temperature patterns are affected by the intensity of the sunlight reaching individual colonies. Another factor aiding in the control of temperatures is the amount of vegetation providing shade in close vicinity to nests' entrances. All of these abiotic conditions affected the density of *T. septentrionalis* colonies within BEP.

The only below ground abiotic factor that consistently influenced the location T. septentrionalis colonies was soil water content. Deeper soil layers contain more water and are more difficult for the ants to move and retain structural integrity. High soil water content may also be detrimental to the survival of the fungal gardens by making them more susceptible to disease. Other than directly influencing *T. septentrionalis*, soil water content functions in the ability of other species of ant and plants to establish themselves. *G. baccata* prefer wetter environments and are therefore more often associated with

areas that are unpopulated by *T. septentrionalis*, *Vaccinium* sp. prefer dryer soils and more commonly associated with areas that are populated by *T. septentrionalis*. This same pattern was found for the ant community where *D. bureni*, *F. subsericea*, *L. alienus*, *P. faisonensis*, *P. vividula*, and *P. pennsylvanica* were never found in the same areas as *T. septentrionalis*, and they all prefer damp nesting sites. Only the generalist *A. treatae* was found to be more common in areas unihabited by *T. septentrionalis*, but no direct competition has been noted in this study, or prior studies.

2) Effect of substrate nutrient and energy content on foraging behavior:

A) Nutrient and energy content of the various types of foraged material used as substrate will not vary significantly. This hypothesis was tested by comparing materials comprising the foraged substrate.

B) Substrate will be selected by *T. septentrionalis* based on ease of collection and availability. This hypothesis was tested by comparing the composition of the materials foraged over the course of the active season compared with available forage material in the surrounding habitat.

Just as the above abiotic factors were found to affect overall activity of *T. septentrionalis* and nest densities, air temperature was also found to influence the rate of substrate collection by individual nest. Even during the prime foraging times such as the early vegetation growing season (when young leaves are readily available and temperatures are optimal for foraging) *T. septentrionalis* brought primarily detrital materials back to their nests. Fresh substrate contained higher nutrient concentrations than detrital substrate, but there was very little difference calorically. This activity

pattern may be explained by the difference in energy expenditure between cutting fresh materials and scavenging detritus.

3) Energy dynamics of the ant-fungus symbiosis: The fungus represents a necessary intermediary in the dietary chain of *T. septentrionalis*. By allowing the fungus garden to process the foraged substrate, *T. septentrionalis* is provided with a higher quality food item, producing a higher assimilation rate than would be expected from direct consumption of foraged material. This hypothesis was tested by comparing the energy and nutrient content of freshly foraged materials and fungal samples.

Most of the substrate that *T. septentrionalis* collects is high in complex carbohydrates that are inaccessible to the ants directly. The enzymatic activities of the fungal gardens provide the readily accessible form of nutrition. However, the easy of accessibility does not translate to a more concentrated nutrient source, but a source of soluble nutrients that are more easily assimilated by *T. septentrionalis*. An additional benefit of the *T. septentrionalis*/fungal symbiont is that nutrients that are not assimilated by either of the two components are returned to the environment in a readily available form.

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Publications

Howell, J.P. 1999. The impact of fertilizer on the uptake of mercury by plants. McNeese State University. Thesis. pp. 54.
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