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## Observations of the turfgrass ant, *Lasius neoniger* Emery (Hymenoptera: Formicidae), in a managed turfgrass setting.

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OBSERVATIONS OF THE TURFGRASS ANT, *LASIUS NEONIGER* EMERY  
(HYMENOPTERA: FORMICIDAE), IN A MANAGED TURFGRASS SETTING

A Thesis Presented

by

SEAN F. WERLE

Submitted to the Graduate School of the  
University of Massachusetts Amherst in partial fulfillment  
of the requirements for the degree of

MASTER OF SCIENCE

February 2000

Department of Entomology

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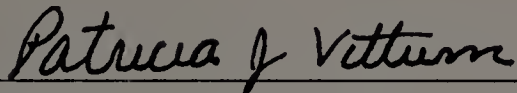
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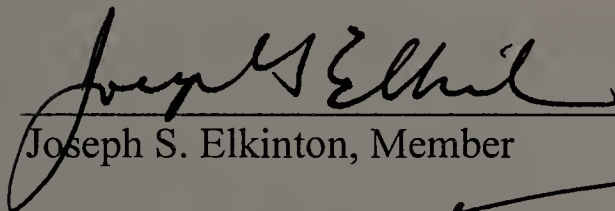
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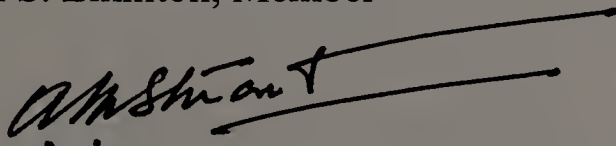
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## ABSTRACT

### OBSERVATIONS OF THE TURFGRASS ANT *LASIUS NEONIGER* EMERY (HYMENOPTERA: FORMICIDAE) IN A MANAGED TURFGRASS SETTING

FEBRUARY, 2000

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The turfgrass ant *Lasius neoniger* was studied along with other insects that occur in manicured turf. The ants were observed over several seasons using pitfall traps and soil core sampling to elucidate their vertical distribution and their distribution with regard to mowing height in manicured turf. A test of turfgrass ants' response to pesticide application was also conducted. The ants were found to be an important presence in closely mowed areas of turf and were also seen to undergo seasonal vertical migration in the soil profile. Some evidence was seen of possible pesticide avoidance behavior.

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## CHAPTER I

### ANTS IN FINE TURF

Ants (Hymenoptera: Formicidae) are among the most numerous animals on the Earth (Hölldobler and Wilson 1990). From a human viewpoint they are among the most conspicuous insects. This ubiquity has important implications when one considers ants in the context of the ecosystems of which they are a part, mainly because of the effects ants exert on the other components of these systems. In ecosystems that have been changed in ways that might affect ants, these effects are important to understand. Such is the case with manicured turfgrass, sometimes referred to as fine turf. In its most extreme manifestation, fine turf can consist of a monoculture of a given turfgrass species that is regularly mowed to a very small fraction of the height to which the plant is best adapted. The reduced mowing height presents a form of stress that often necessitates very high levels of maintenance (notably inputs of water and nutrients). Meanwhile, the ability of that turfgrass to resist damage from insects or other organisms is greatly limited. The ecological place of ants in this environment might be very important considering their predatory nature, since the majority of ants prey on insects or other arthropods to some extent (Wilson 1971).

All ants are eusocial, the highest degree of social organization found in the insects and described by Wilson (1971) as social organization exhibiting three defining traits. There must be a reproductive caste separation (non-reproductive individuals must exist), cooperative brood care, and the generations must overlap. This lifestyle seems to confer a considerable competitive advantage to insects that have developed it because in most cases, eusocial insects are very successful and highly diverse.

In the northeastern United States the most common ants belong in the subfamilies Formicinae and Myrmecinae. (Other subfamilies are also represented, but are extremely rare in turfgrass). The two subfamilies are quite different, and the Formicinae are considered the more evolutionarily advanced of the two (Hölldobler and Wilson 1990). The myrmecine ants still retain a sting derived from a modified ovipositor, and there are two reduced segments, the petiole and the post-petiole, separating the metasoma (the apparent thorax) and the gaster (the apparent abdomen). In contrast, the formicine ants have lost the sting and in its place is a structure called the acidopore, which is an outlet for exocrine gland secretions that serve a number of purposes. The reduction of the third abdominal segment to form the post-petiole is also lost in this subfamily, and only one reduced segment, the petiole, separates the metasoma and the gaster.

Ecologically the two subfamilies are fairly similar. Both subfamilies exhibit a high level of social organization, and nest sizes in some species can exceed 100,000 individuals. Predation on live arthropods is somewhat more prevalent in the Myrmecinae, where the sting is well evolved as an offensive weapon. Formicine ants are more likely to be scavengers or “farmers” (tending aphids or other homopterans as a food source), although predation also occurs. The main focus of this thesis, the formicine ant *Lasius neoniger*, exhibits all three of the traits mentioned above, scavenging, farming, and predation.

The turfgrass ant, *Lasius neoniger* Emery, has become a serious pest for turf managers in the northeast within the past 15 years or so. Previously, this ant was seldom cited as a problem on turfgrass. The reasons for this shift in pest status are presently

unclear, but one very likely cause is the evolution of golf course mowing heights to much shorter cuts, resulting in insufficient turf to mask ant activity. In addition, turf grown at lower heights is less able to recover from stress or insect damage.

Another reason turfgrass ants have reached pest status may relate to recent changes in insecticide use patterns beginning with the banning of organochlorine insecticides such as chlordane in the 1970s. Organochlorines had been used extensively to control white grubs and other pests on turf (even crabgrass) and may have been controlling turfgrass ants secondarily. This secondary control could have been due to direct mortality in the ants, or it may have been a result of the organochlorine eliminating the ants' prey or killing the aphids that they tend on the turf roots. Many of the organochlorines were highly persistent, and treated areas may well have retained insecticidal activity for several years after the last applications were made. Since the ban allowed the continued use of stockpiled organochlorines, applications may have occurred as recently as the early 1980s in some locations.

Whatever the cause, turfgrass ants are now among the most serious pests of managed turfgrass in the northeastern United States, costing turf managers millions of dollars per year in insecticides directed toward their control.

Turfgrass ants damage turf indirectly by excavating soil from nest galleries below the root zone. Much of the current understanding of turfgrass ant ecology is derived from studies in pastures or corn fields, where the dynamics are similar but the ants do not cause significant crop losses. In fact the opposite is probably true, turfgrass ants have been cited as a possible factor in excluding the red imported fire ant from

pastures (Bhatkar et al. 1972) and have been seen to reduce corn pest populations, reportedly by egg predation (Kirk 1981, Ballard and Mayo 1979)

The ants nest in extensive networks of small (1-3mm in diameter) tunnels interspersed with larger galleries which can exceed 15mm across. This tunnel network can extend to a meter or more below the soil surface (Wang 1993, Wang et al. 1995b) and each individual nest typically will have multiple openings to the surface (Wang et al. 1995a). Traniello (1983) recorded as many as 5 distinct colonies inhabiting a square meter of surface area. The result is a situation where large amounts of soil are being transported to the surface and piled in small rings around the nest entrances. These soil modifications can be beneficial, increasing water flow and aerification (Wang et al. 1991, 1996) or they can be detrimental, causing soil desiccation and resultant plant damage. Turfgrass ant activity peaks in mid summer, which complements the aerification activity of another natural soil modifier, the earthworm *Lumbricus terrestris*. Earthworms are most active at a soil temperature of 10°C (Daughberger 1988) and thus in New England are most active in the spring and fall (Brady 1974).

Turfgrass ants however, often cause more problems for turf managers than they solve. The small piles of soil can become numerous enough to be unsightly and, perhaps more importantly, can damage turf maintenance equipment. This damage, in the form of dulled mower blades and clogged rollers, is the primary reason cited by turf managers for the desire to eliminate these ants from areas of closely mowed turf, most typically golf course fairways and tees. Turfgrass ant nest openings on turf maintained at golf course fairway conditions can disrupt the playability of the surface (Figure 1). This type

of damage is deemed unacceptable by many golfers, so turf managers are forced to seek management strategies that will reduce mound-building activity.



Figure 1: Damage caused by turfgrass ants on a golf course. A: an infested fairway. B: close-up of ant nest entrances. Keychain is included in both images to indicate scale.



This thesis presents the results of several studies that looked at various aspects of turfgrass ant behavior. In Chapter Two data are presented that shed light on the physical location of turfgrass ant foragers in relation to mowing height differences present in managed turf, as well as on the spatial distribution of some other selected turfgrass inhabiting predators.

In Chapter Three the results of a study of the vertical distribution of turfgrass ants in the soil are presented. This investigation was designed to examine the vertical movement of ants in the soil profile throughout the growing season.

Chapter Four presents the results of a study which looked at the response of turfgrass ants to an insecticide application. The same vertical sampling technique that was used for the depth study presented in Chapter Three was used in this study, revealing considerable information regarding the species' response to a surface application of an insecticide.

## CHAPTER II

### ANTS AS POTENTIAL PREDATORS IN MANICURED TURFGRASS

#### Introduction

The fact that ants, *Lasius neoniger* in particular, are pests when they occur in manicured turfgrass settings is undeniable (Thompson 1990). They cause maintenance problems as discussed in the previous chapter and, once established, they tend to expand their colonization within the turfgrass habitat (Wilson and Hunt 1966). They have also been cited as house invaders (Smith 1965). Simply eliminating turfgrass ants from these sites, however, could well result in secondary pest outbreaks. Most currently known formicid species are predatory at least to some extent (Hölldobler and Wilson 1990). Turfgrass ants have been observed consuming the eggs of cutworms (Lepidoptera: Noctuidae), a serious turf pest (Lopez-Gutierrez, pers. comm.), as well as the eggs of the western corn rootworm, *Diabrotica vergifera* (Coleoptera: Chrysomelidae) (Ballard and Mayo 1979). Reductions in corn rootworm population density have been associated with the presence of *L. neoniger* (Kirk 1981). It is reasonable to assume that egg predation of this sort would extend to other turf pest species such as Scarabaeid beetles.

Turfgrass ants have also been shown to be highly competitive against *Solenopsis invicta*, the red imported fire ant (Apperson and Powell 1984, Bhatkar et al. 1972, Bhatkar 1973, 1988, Showler and Reagan 1987, Vinson and Greenburg 1986, Vinson 1994) and have been suggested as a possible factor in limiting the northward spread of this notorious stinging pest (Whitcomb et al. 1973, Buren et al. 1974). Though turfgrass ants are primarily scavengers and aphid tenders, their predatory potential has been established in numerous prior studies (Ayre 1963, Hasse 1971, Paulson and Akre 1992,

Traniello 1987). Turfgrass ants have also been reported feeding directly on plant nectar (Fritz and Morse 1981), but this probably represents a negligible contribution to colony nutrition in the fine turf environment.

The observations described in this chapter were conducted in order to establish the location of turfgrass ants as a predatory presence in turfgrass mowed at fairway (intermediate) and “rough” (high) heights in a golf course setting and to compare the distribution of turfgrass ants with that of other predatory insects. The working hypothesis was that *Lasius neoniger* represents an important member of the guild of predators present in manicured turfgrass. This idea is supported by the overall trap data which is presented in Appendix A, Table 2. There it can be seen that of over 18,000 insects captured in golf courses about 37% were *Lasius neoniger*.

### Methods

Golf courses are typically arranged with a relatively closely mowed area (12-30 mm), the fairway, surrounded by an area which is allowed to grow much taller (50-130 mm), the rough. The line separating these areas is sharply defined as a result of repeated mowings at the prescribed height, and remains relatively constant in space for several years at a time.

Following the procedures of Smitley et al. (1998), a series of pitfall traps was set in a line at right angles to this rough/fairway demarcation line. Four sets of seven traps each were installed at each of three sites in 1996 and at one site in 1997. Traps were made from 45 ml glass vials inserted into holes in the soil. Holes for the traps were cored using a 2.54 cm diameter soil sampling tool and the traps were inserted so that the surface of the glass vial was flush with the soil surface. The center trap was installed on

the rough/fairway border and three traps were installed on either side of the border separated by 4.57 meters in the direction of the trap line. The four trap lines were separated by a distance of 15.2 meters along the rough/fairway demarcation line. A schematic drawing of the trap layout is shown in Figure 2.

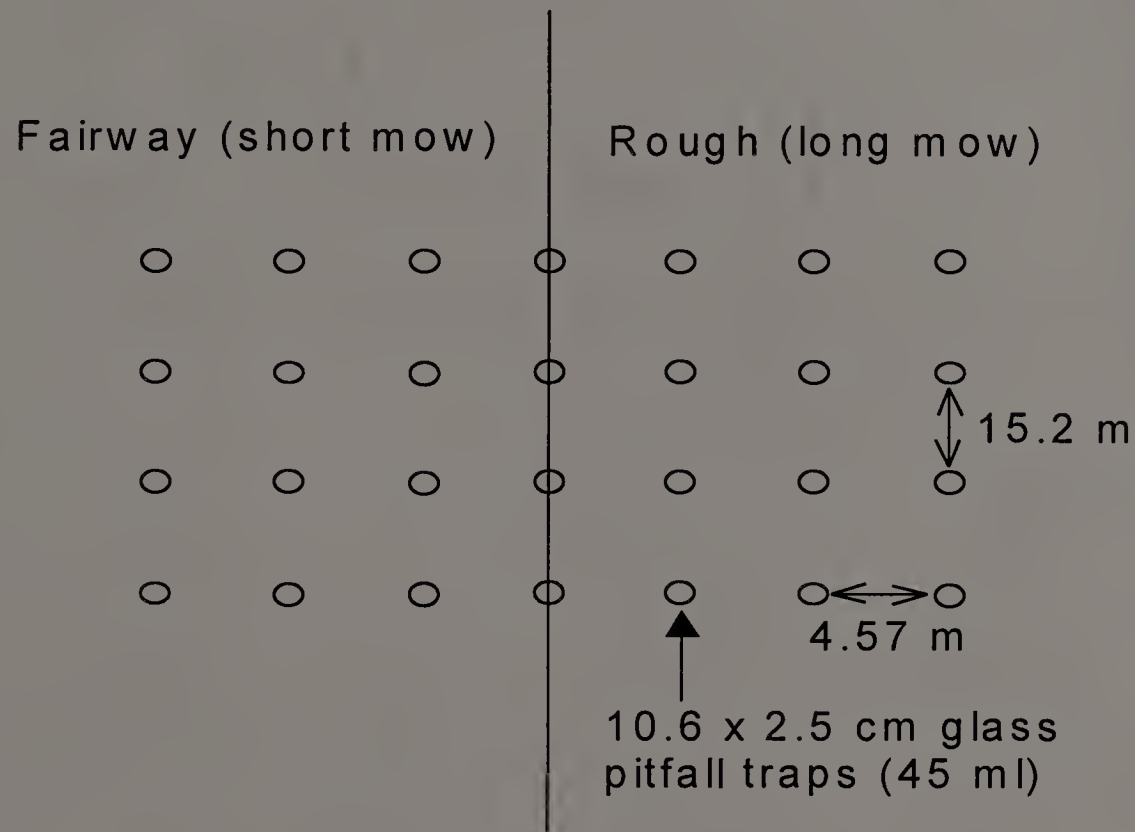


Figure 2: Pitfall trap layout (not to scale). The area to the left represents the fairway, which is mowed at approximately 15 mm, the area to the right represents the rough, which is mowed at approximately 75 mm. The borderline separating the two mowing heights is in the center of the diagram.

Each trap was filled to a depth of 3 cm with ethylene glycol (automotive antifreeze). The trap contents were recovered and the traps refilled semi-regularly throughout the summer. The intended sample interval was 7 days but occasionally varied slightly depending on golf course tournament schedules and the logistics involved in travelling to the widely separated sites. These samples were then stored in a commercial freezer at  $-20^{\circ}\text{C}$  for later identification and analysis.

Identifications were made to various taxonomic levels. For example, ants were identified to subfamily, genus (Bolton 1994) or species (Creighton 1950, Wilson 1955), non-insects were identified to order or class, and most other insects were identified to family. Some taxa, such as Collembola and mites (Acarina), were too numerous to include. In similar studies of the effect of insecticides on non-target arthropods, samples from fine turf regularly have yielded as many as 4,000-6,000 Collembola per square meter (Vittum 1996, unpublished data). Additionally, Collembola and similar taxa were not considered to be within the guild of predators being studied, so data on these were not collected. Diptera that were collected in the traps also were considered to be outside the scope of the study and were not identified below order.

A simple diversity index was calculated for these data that consisted of the mean number of different taxa per trap-day. For example, a mean of 4 different taxa in traps that had been set for 7 days would yield a diversity index of  $\frac{4}{7}$  or 0.57. Admittedly, this extremely simplified index suffers from some drawbacks. The most obvious shortcoming is that as time increases the number of new taxa being trapped will decrease. This is not, however, a problem here since the different sites are not being compared to each other but rather comparisons are being made within sites, where the sampling interval was always the same.

In 1996 this pitfall trap array was placed on one fairway/rough location at each of three golf courses that had experienced substantial ant activity in previous years: Stockbridge Country Club in Stockbridge, Mass., Worcester Country Club in Worcester, Mass., and Hickory Ridge Country Club in Amherst, Mass.. The same study was repeated in 1997 at Stockbridge Country Club only.

## Results

Overall, 18,184 arthropods were collected and separated into 58 distinct taxonomic categories (see Table 2, Appendix A). Many of these categories contained multiple species, thus the turfgrass ecosystem sampled was found to contain a fair diversity of species, probably over 150. Of the overall trap capture of 18,184 specimens the most common were turfgrass ants (37%), which were slightly more abundant than staphylinid beetles (31%). The families Staphylinidae and Formicidae were by far the most dominant predatory groups seen in this study, making up 77% of the arthropods trapped.

In 1996, 8167 arthropod specimens were collected from the traps at the Stockbridge Country Club, Stockbridge, Mass. (hereafter designated SBCC) and identified. Worcester Country Club, Worcester, Mass. (hereafter designated WCC) yielded 2046 specimens and the Hickory Ridge Country Club, Amherst, Mass. (hereafter designated HRCC) yielded 2644 specimens. In 1997 the SBCC traps contained 5327 specimens. The overall trap contents are presented in Figures 3-6. Appendix A includes a list and brief description of the various taxa collected throughout.

# Hickory Ridge CC, 1996 trap contents

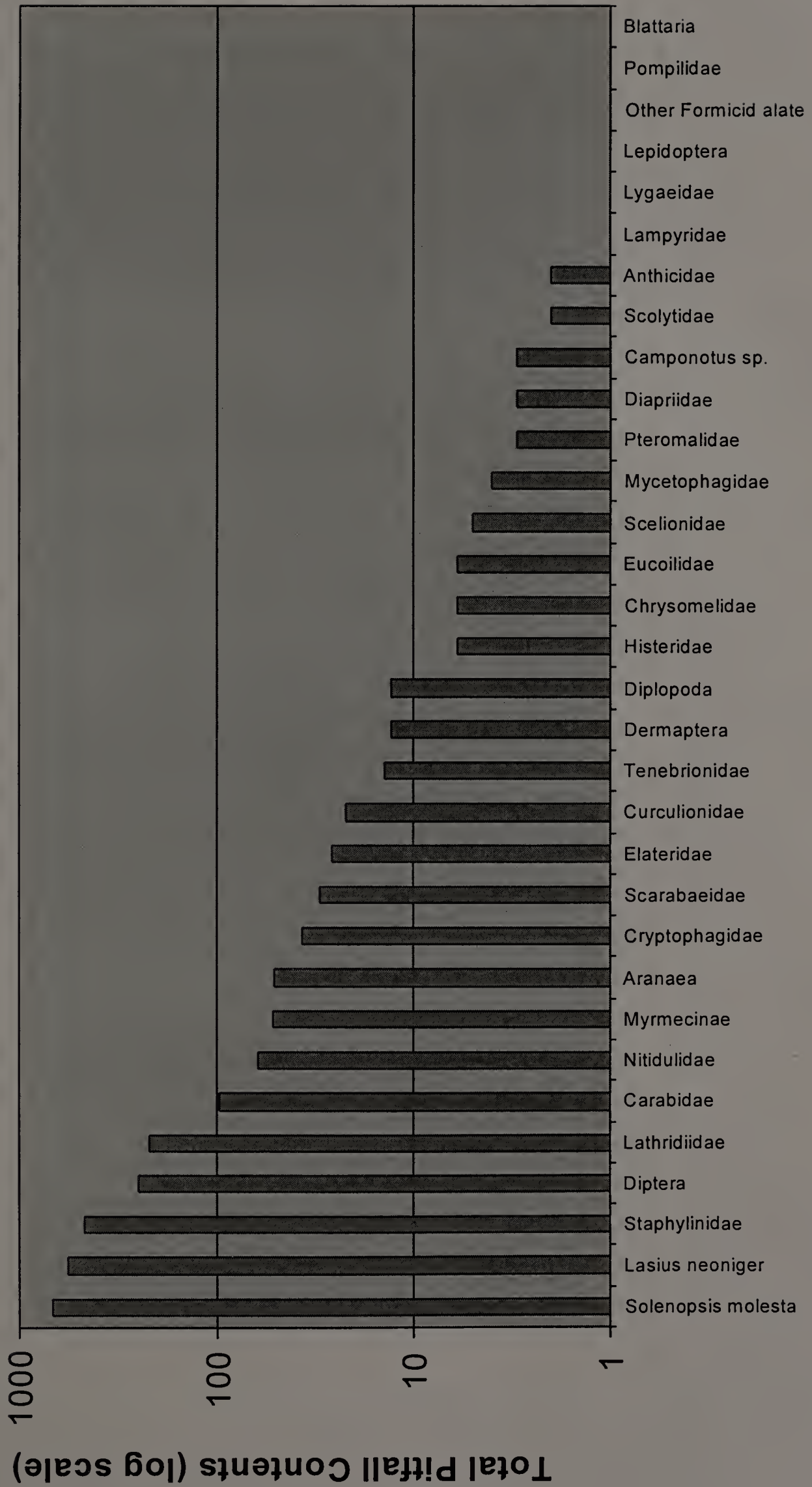


Figure 3. Complete pitfall trap contents for the Hickory Ridge Country Club in 1996. Logarithmic scale.

# Worcester CC, 1996 trap contents



Figure 4. Complete pitfall trap contents for the Worcester Country Club in 1996. Logarithmic scale.



# Stockbridge CC, 1996 trap contents



Figure 5. Complete pitfall trap contents for the Stockbridge Country Club in 1996. Logarithmic scale.

# Stockbridge CC, 1997 trap contents



Figure 6. Complete pitfall trap contents for the Stockbridge Country Club in 1997. Logarithmic scale.

## Taxonomic diversity

The SBCC data were the most diverse with 41 different taxa collected in 1996 and 45 in 1997, HRCC had 32 trapped taxa and WCC had 28.

A diversity index ( $d$ ) consisting of the number of taxa per trap day was calculated for all traps. These data are presented in Figures 7-10.

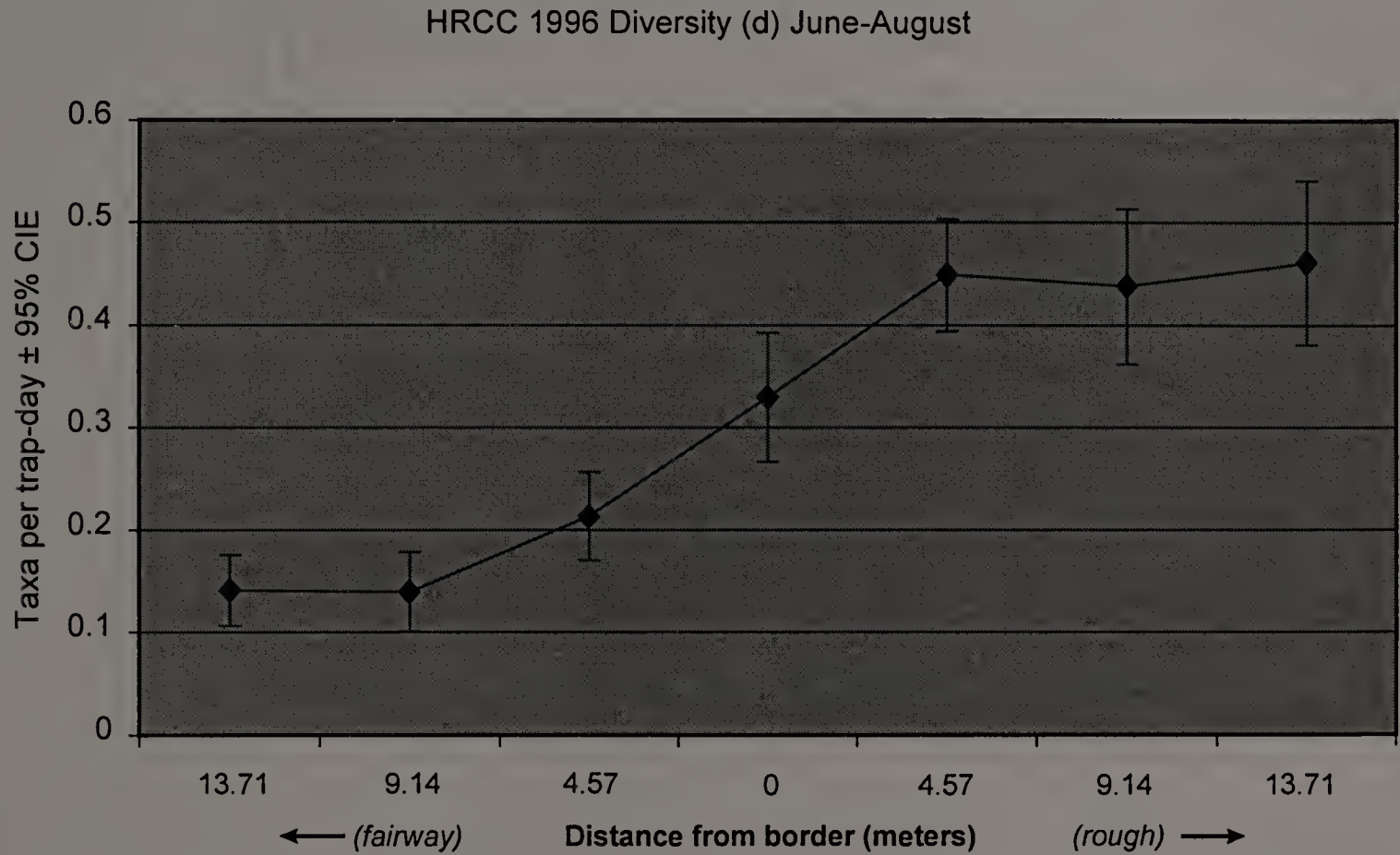


Figure 7. Mean diversity indices for HRCC 1996.

Diversity index (d) WCC 1996

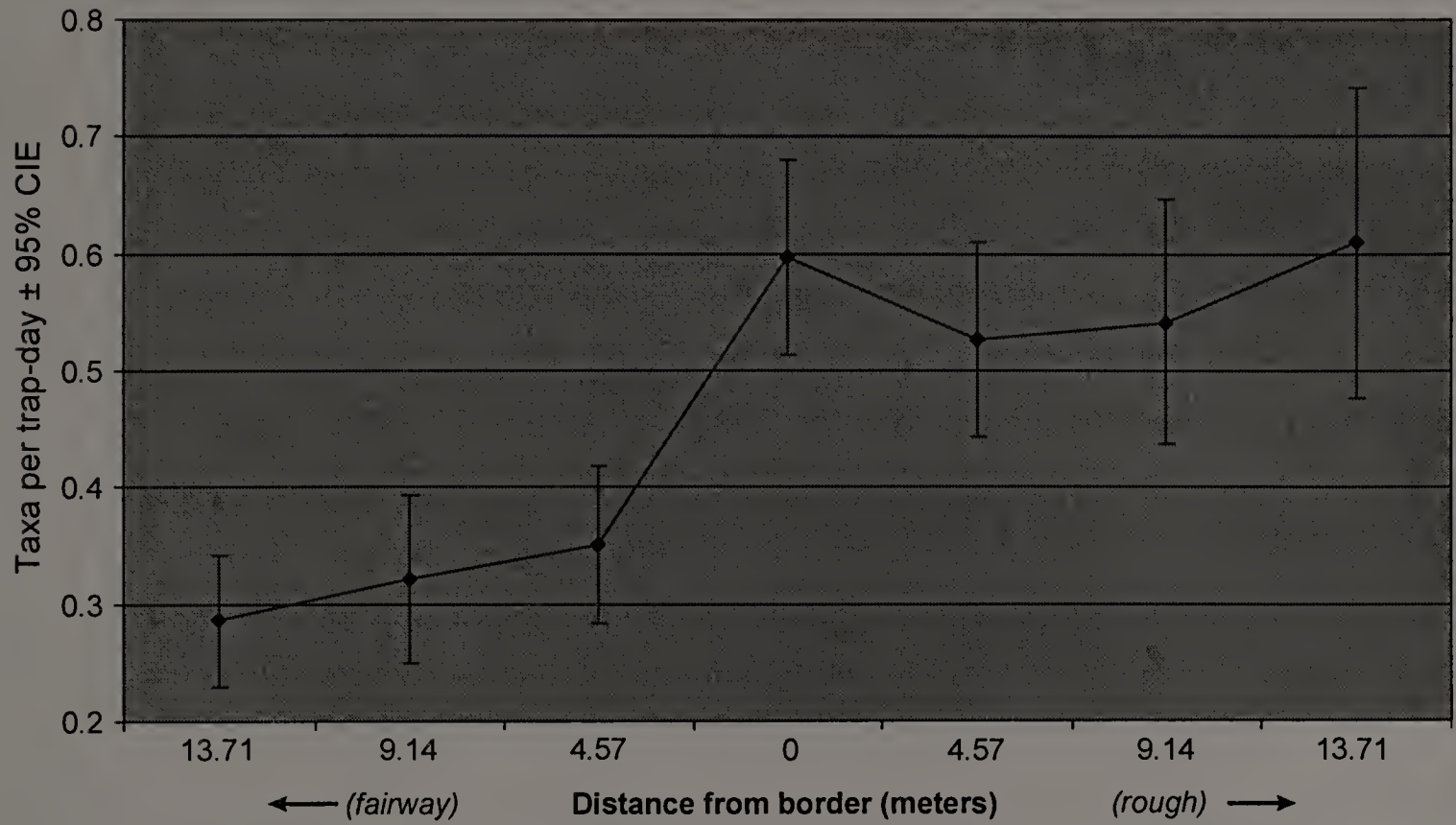


Figure 8. Mean diversity indices for WCC 1996.

Diversity index (d), SBCC 1996

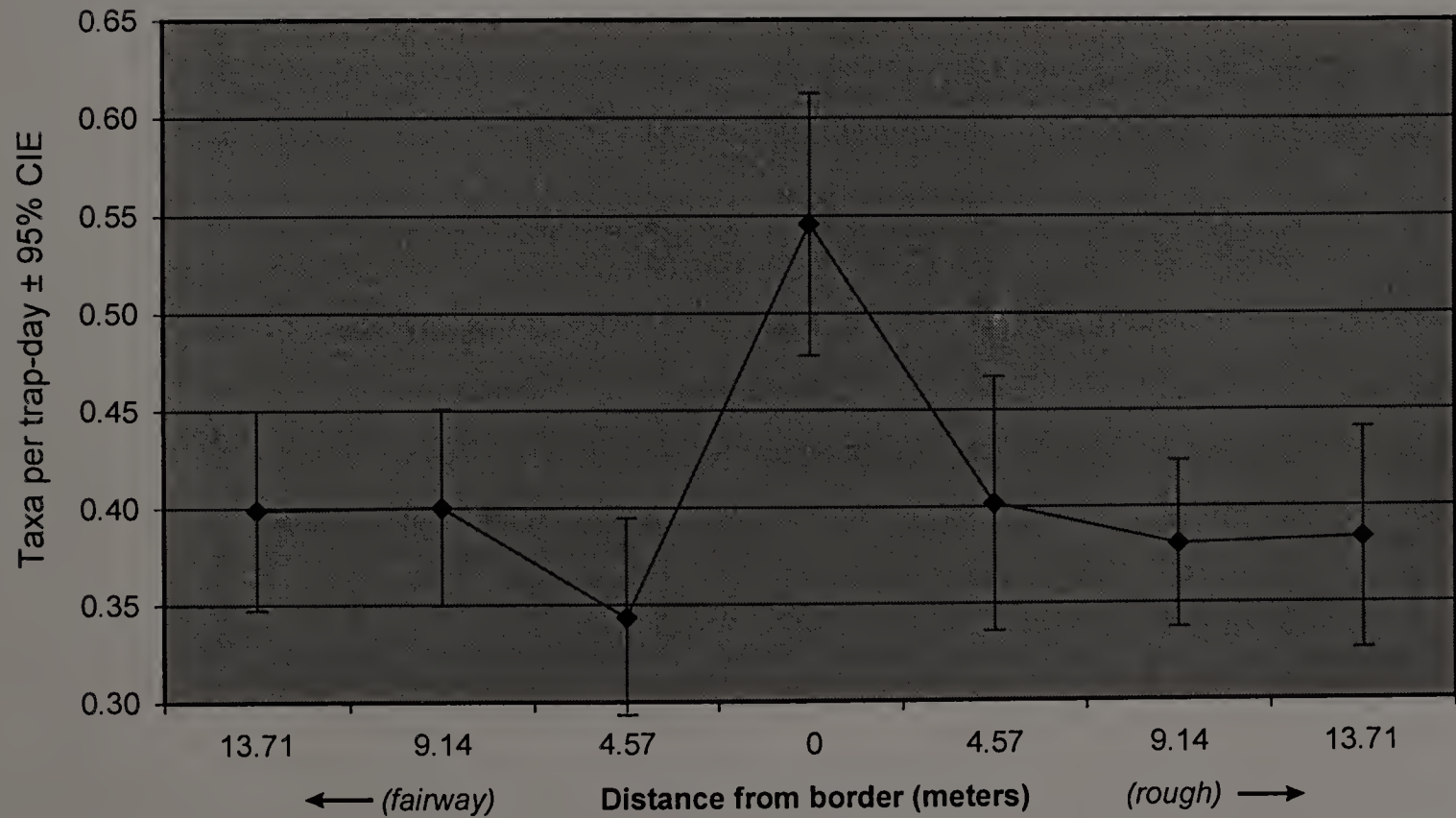


Figure 9. Mean diversity indices for SBCC 1996.

Diversity index (d), SBCC 1997

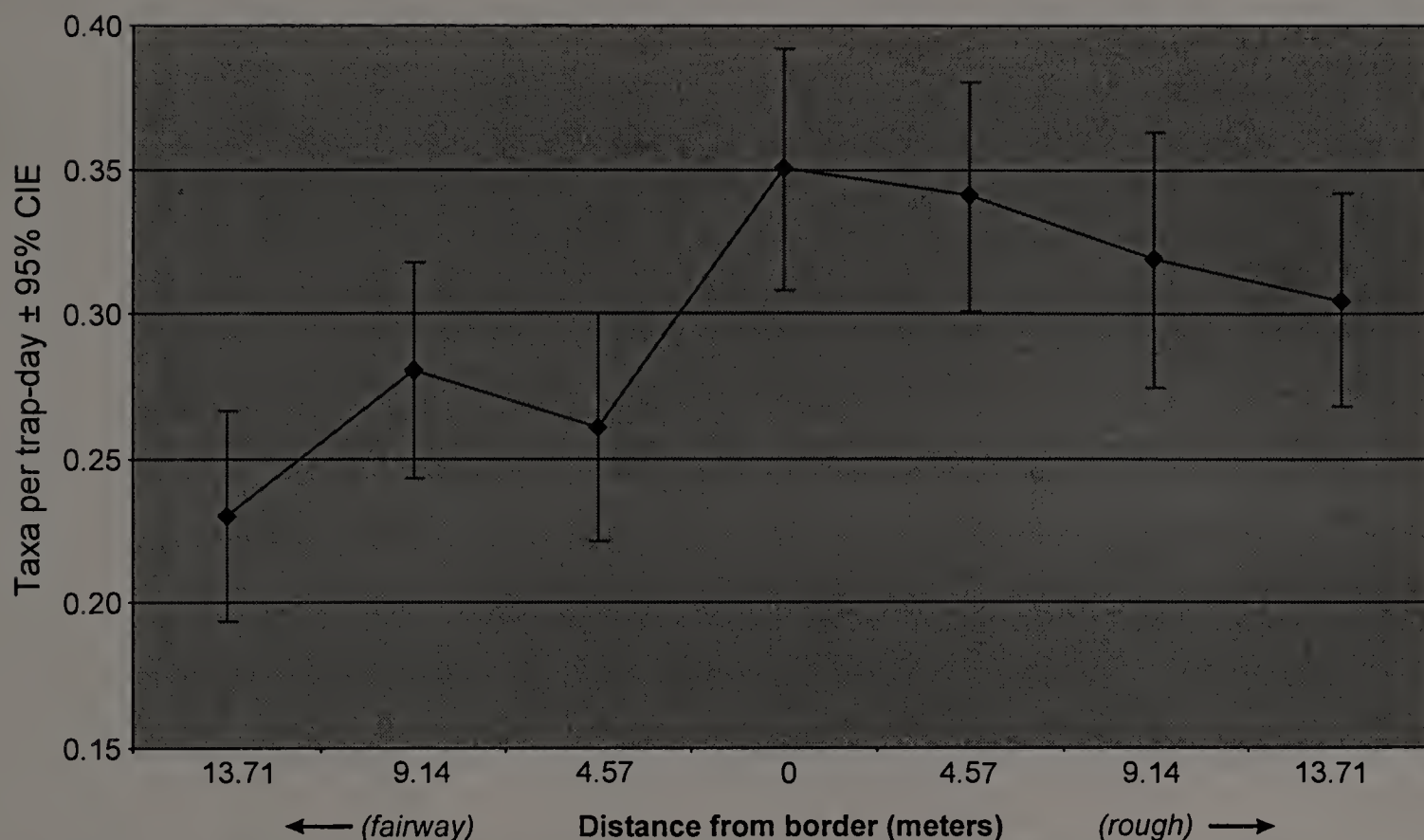


Figure 10. Mean diversity indices for SBCC 1997. Error bars represent the limits of the 95% confidence interval estimates for each mean.

Predator abundance

For the purpose of analyzing these data, only the guild of predators that actively forage for prey on the turf plants and the ground were considered. These included the ant species *Lasius neoniger*, *Solenopsis molesta* and *Myrmica americana*; the predatory beetle families Staphylinidae, Carabidae and Histeridae; and various spiders, Order Aranaea. Figures 11-14 show the relative abundance of these 7 taxonomic groups of predators as they varied in relation to the location of the trap. Trap location is indicated on the x-axis and corresponds to the pitfall layout previously shown in Figure 2.

Trap capture vs treatment, HRCC 1996

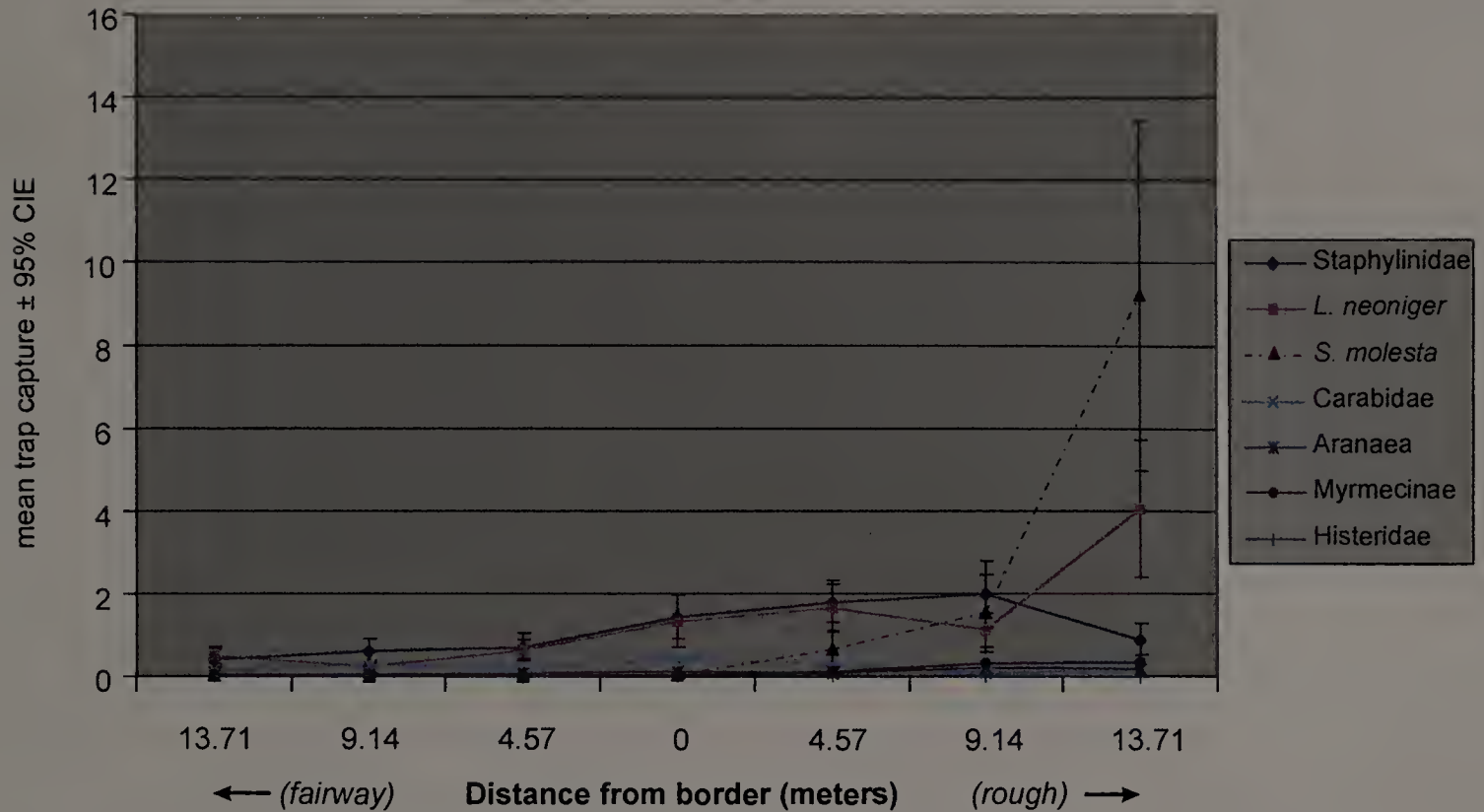


Figure 11. Predator abundance versus treatment, HRCC 1996 (n=60). Error bars are calculated for the three dominant predatory taxa, turfgrass ants, thief ants, and Staphylinid beetles.

Trap capture vs treatment, WCC 1996

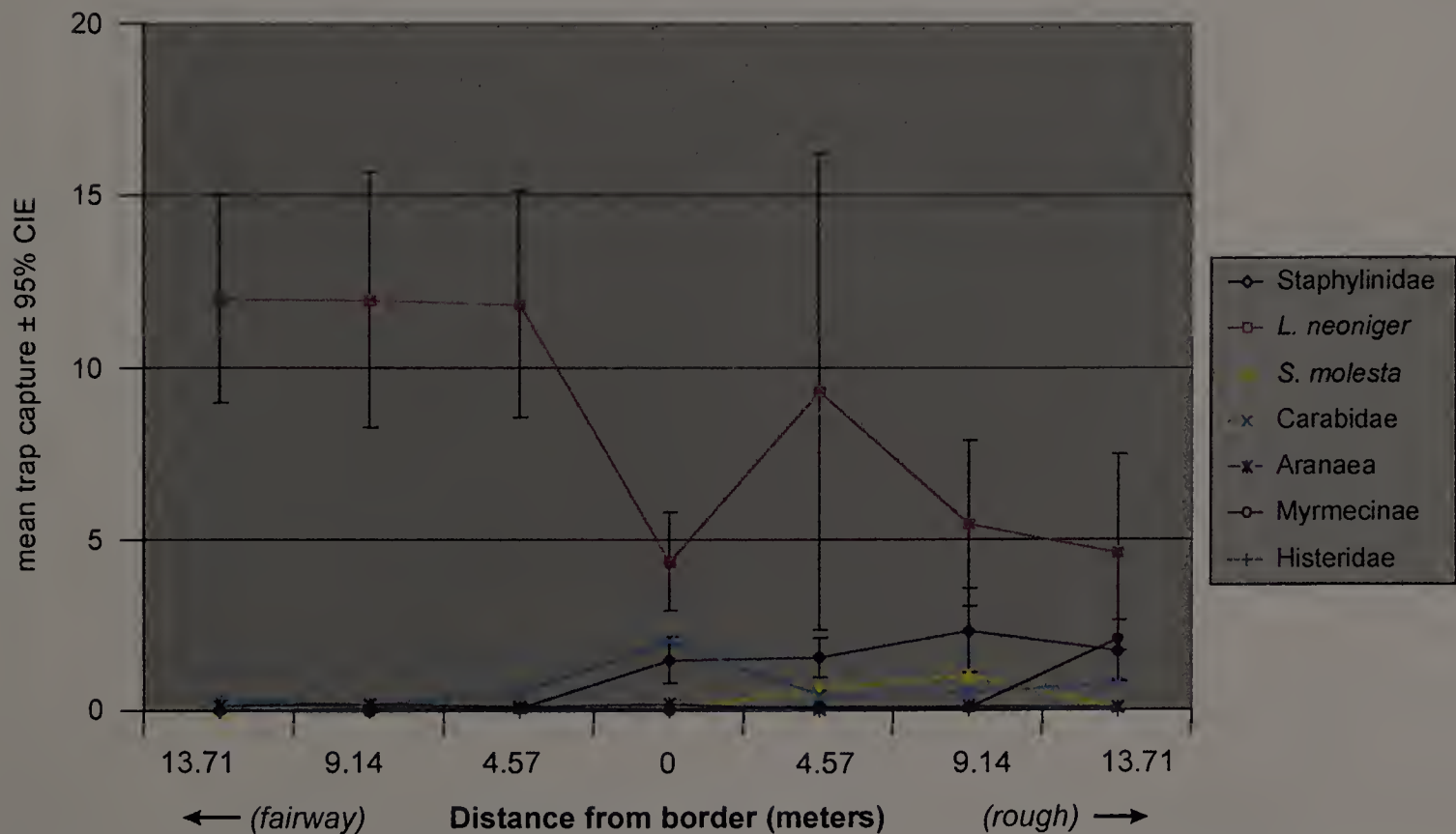


Figure 12. Predator abundance versus treatment, WCC 1996 (n=24). Error bars are calculated for the two dominant predatory taxa, turfgrass ants and Staphylinid beetles.

Trap capture vs treatment, SBCC 1996

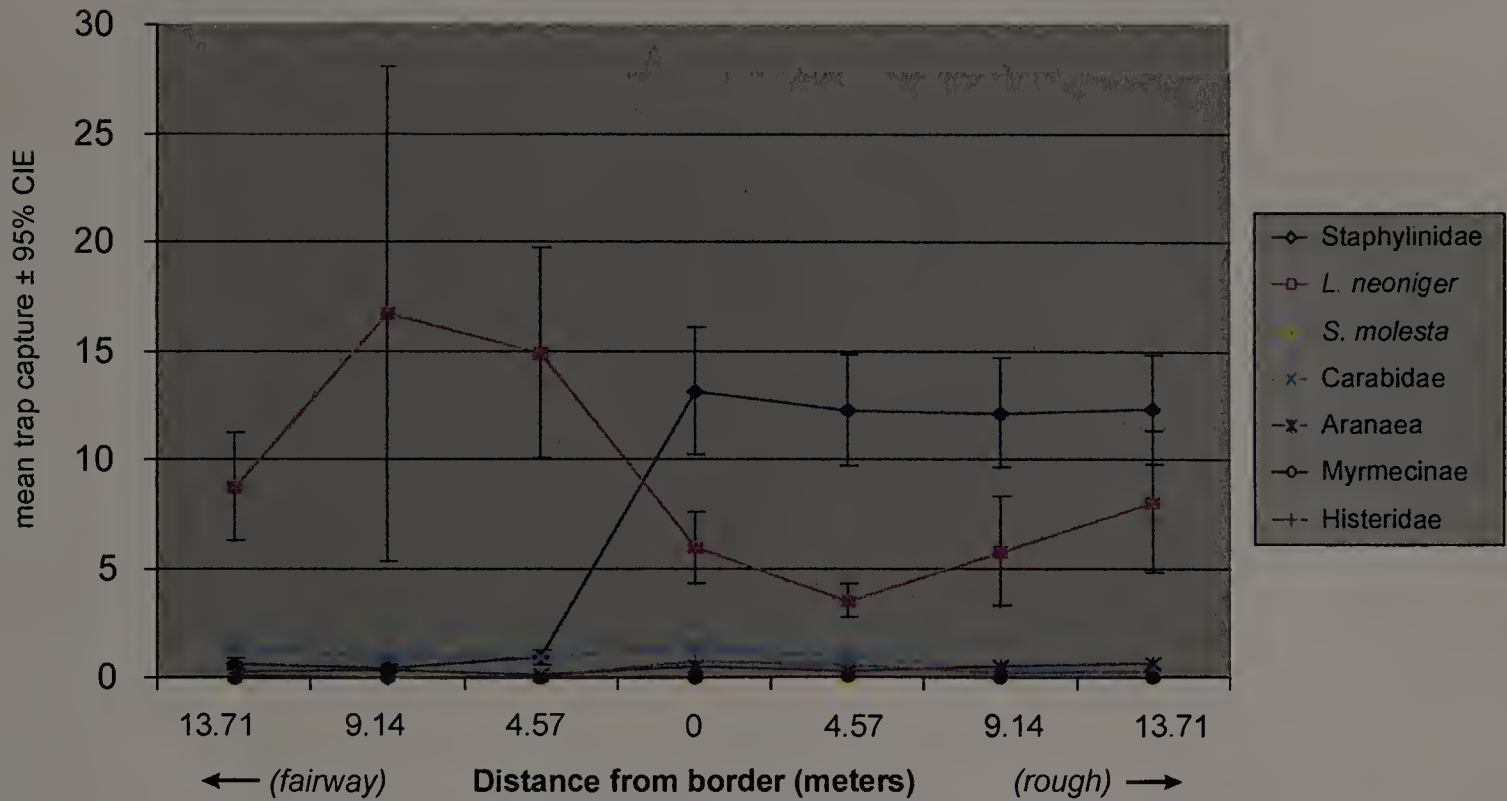


Figure 13. Predator abundance versus treatment, SBCC 1996 (n=60). Error bars are calculated for the two dominant predatory taxa, turfgrass ants and Staphylinid beetles.

Trap capture vs treatment, SBCC 1997

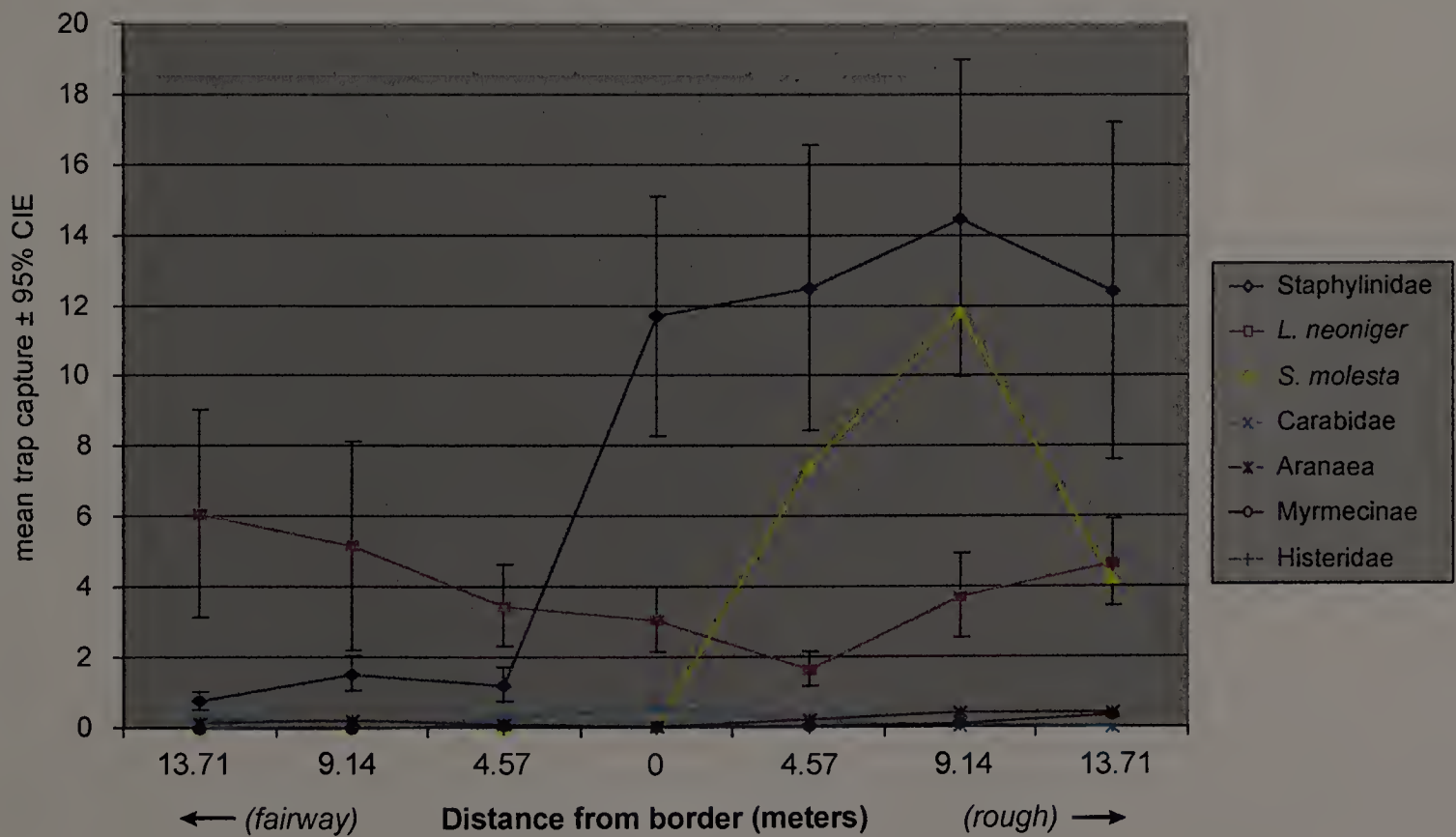


Figure 14. Predator abundance versus treatment, SBCC 1997 (n=36). Error bars are calculated for the two dominant predatory taxa, turfgrass ants and Staphylinid beetles.

These data seem to show an inverse correlation with insecticide application data from the three sites when they are compared as shown in Table 1. Based upon conversations with golf course personnel, we know that the insecticide loads at these sites were as follows; SBCC, **LOW**: one application of Merit™ (a formulation of imidacloprid targeting white grubs) at 0.3 lbs. AI/acre in August 1996, no insecticides were applied in 1997; HRCC, **MEDIUM**: two applications of Dursban 50W™ (a general purpose organo-phosphate insecticide targeting cutworms [Lepidoptera: Noctuidae]) at 4 lbs. AI/acre one in June 1996 and again one in July 1996; and WCC, **HIGH**: Dursban at 2 lb AI/acre in May 1996, Merit at 0.3 lb AI/acre in June 1996, and Turcam (a formulation of bendiocarb, a general purpose organo-phosphate targeting cutworms and white grubs) at 3lb AI/acre in July 1996. These applications were made to fairways only, so none of the pitfall traps located in the rough would have been in treated areas. The numbers for SBCC represent the mean of the two years sampled. The diversity referred to is simply the total number of taxa collected, again a mean for SBCC.

Table 1: Pitfall trap capture and diversity compared to insecticide application level.

Explanation of insecticide application level is contained in the text.

	<b>Insecticide application level</b>	<b>Total trap capture</b>	<b>Diversity</b>
<b>SBCC</b>	LOW	6747	43
<b>HRCC</b>	MEDIUM	2644	32
<b>WCC</b>	HIGH	2046	28



Thus far, the point is moot because turfgrass ant activity has persisted despite repeated efforts by turf managers to reduce ant populations. Chapter IV will consider possible explanations of this phenomenon.

### Discussion

The working hypothesis is that *Lasius neoniger* represents an important predatory member of the ecological community present in manicured turfgrass. This hypothesis was supported by the data.

It can be seen in Figures 3-6 that turfgrass ants were always among the most numerous insects collected, dramatically outnumbering most other taxa found in the traps. This isn't surprising. Cockfield and Potter (1984) found turfgrass ants to be the dominant insects in lawns in Kentucky and they are often dominant in other habitats where they are found. Regardless of golf course sampled, the two most numerous predators were turfgrass ants and Staphylinid beetles. The thief ant (*Solenopsis molesta*) also appears to be present in substantial numbers. However, this could be considered to be an artifact of our experimental design. There was large variance in the data for thief ants because they tended to appear in very large numbers in traps that were located near their nests, but were absent from more distant traps.

Myrmecine ants, spiders, and histerid beetles were far less common and carabid beetles were intermediate in occurrence. In the case of the spiders this absence from the traps is also likely to have been an artifact of our sampling methods- the small diameter of the pitfall traps undoubtedly precluded the larger hunting spiders from being trapped.

The rest of the data in this chapter focus on the mowing height differences present in the golf course environment. There are two areas being considered, the

fairway, where traps were given a negative number designation and the mowing height is approximately 8-15 millimeters, and the rough, with positively numbered traps and a mowing height of over 50 millimeters. One thing that is important to note is that these areas appear to be distinct; the distance from the border showed no significant effect (as reflected in the overlap of confidence intervals shown in Figures 7-14) on either diversity or predator abundance. This implies that species that live in the rough do not make forays into the fairway, and foragers that live in the fairway seldom venture into the rough. The two habitats are more distinct from each other than had been expected.

#### Taxonomic diversity

Figures 7-10 show the effect of mowing height on taxonomic diversity in the golf course environment. Diversity tends to be greatest on the border between the two habitats, and higher in the tall grass than in the fairway. This phenomenon would be even more apparent if the data were corrected to account for surface area sampled; the tall grass provides far more surface area than the short-mowed fairway, and thus trap captures were most likely attenuated in the rough. If even a modest correction factor were included to account for this disparity, the diversity in the tall grass (rough) would always be significantly greater than that in the fairway.

#### Predator abundance

The next series of graphs, Figures 11-14, show where seven selected predatory taxa (*Lasius neoniger*, *Solenopsis molesta* and *Myrmica americana*; the predatory beetle families Staphylinidae, Carabidae and Histeridae; and the spiders, Order Aranaea) occur in the fairway and the rough. At HRCC insects (Figure 11) were fairly sparse, with predator capture increasing in the rough, though not always significantly and never

dramatically. The remaining graphs, however, are more striking. The relative importance of *Lasius neoniger* in the short grass is clear in both the WCC data and both years of SBCC data. Turfgrass ants were always significantly more numerous (as indicated by the 95% confidence interval estimates calculated and shown as error bars on the charts) than any of the other predatory species in the fairway. At Stockbridge, turfgrass ant and staphylinid abundances were inverted with respect to habitat type; turfgrass ants were significantly dominant in the fairway, while staphylinids were significantly dominant in the rough. At Worcester, the staphylinid numbers increased in the rough, but turfgrass ants were still more numerous. At both of these sites, even in the rough, turfgrass ants always outnumbered the other predators significantly. These results are similar to the findings of Smitley et al. (1998) and Rothwell and Smitley (1999), who found an inverse relationship between predatory insect species (which were more common in the rough) and a pest beetle, *Ataenius spretulus* (Coleoptera: Scarabaeidae) (which were more common in the fairways).

Since many pest control efforts on a golf course are directed at the fairway, but few or no pesticides are applied to the roughs, these data suggest that turfgrass ants are an important factor to be considered in the context of pest management. While they can be pests themselves, the ants are also beneficial, and the consequences of removing them should be studied more intensively before turf managers embrace disruptive management strategies such as insecticide applications too aggressively.

## CHAPTER III

### TEMPORAL AND SPATIAL BEHAVIOR PATTERNS OF TURFGRASS ANTS

#### Introduction

The spatial distribution of *Lasius neoniger* has been well studied (although not in fine turf) at the two dimensional soil surface (Levings and Traniello 1981, Traniello 1983, 1989a). Traniello and Levings (1986) used intercolony aggression to establish the colony affiliation of individual nest entrances. They found that nest entrances are overdispersed within colonies and are separated on average by about 38 cm, approximately twice the optimal distance for workers retrieving prey. They also found that workers using a given nest entrance tended to use the same entrance repeatedly, even preferentially over other, closer entrances to its path.

Worker ants often use hindgut pheromone trails to recruit other workers to food, which mainly consists of living or dead arthropods (Traniello 1989b). Traniello (1983) reported that about 85% of the total biomass of prey retrieved by *Lasius neoniger* foragers was cooperatively retrieved by groups of several to many workers. Both short-range and long-range pheromones were used to recruit workers to prey.

All of these studies were conducted in either abandoned pastures or cornfields, though the two dimensional pattern of nest entrance dispersal appears similar in manicured turf.

Very little research has been devoted to the subsurface spatial dynamics of this insect. Because of the turfgrass ant's pest status, these subsurface dynamics are important to understand. Pesticide applications that are made when most ants are deep in the soil profile (>5 cm) are essentially wasted because most insecticides applied to

established turf do not reach the soil. Understanding how the ants move in the soil over time is critical to avoid such inefficiencies in control methods.

The objective of this study was to determine the movement patterns of *Lasius neoniger* in the top 38 cm of the soil profile of a lawn-type turfgrass throughout the growing season.

### Methods

This study was conducted on a 50 meter square plot of mowed turfgrass at the University of Massachusetts Turf Research Facility in South Deerfield, Massachusetts. The plot was maintained like a typical lawn (Kentucky bluegrass/perennial ryegrass blend), established in 1989, and mowed weekly at a height of 5 cm. Sampling was randomized within the plot by the following method: a random starting point was chosen by throwing a coin into the plot and taking the first sample from the point at which the coin landed. Subsequent samples were located by flipping the coin and taking ten paces in the direction of the top of the embossed image on the coin (be it “heads” or “tails”). If this line intersected the edge of the plot, the line was reflected from the edge with the angle of reflection equal to the angle of incidence. Twenty samples were taken on each sample date.

Samples were collected roughly monthly from July to October of 1996 and then biweekly from April to November of 1997. Samples were also collected in December of 1997 and March of 1998. Samples were always collected within 1.5 hours of 12:00 noon (clock noon) in order to minimize diel artifacts in the data.

A standard golf course “cup-cutter” (Figure 17A) was used to cut a soil core 107 mm in diameter by 150 mm deep. This process was repeated in the same hole three

times so that a total depth of 380 mm was attained. Each sample was then subdivided into 76 mm depth increments to yield 5 individual cores per sample. Each core was placed in a plastic bag and labeled as to sample number, depth of core, and sample date, and all samples were taken back to the laboratory and frozen at -20°C in a commercial freezer to euthanize and preserve the ants. The sampling procedure is shown below in Figures 15-17.



Figure 15: Sampling method. Study site in South Deerfield with sample bags on mixed turfgrass.

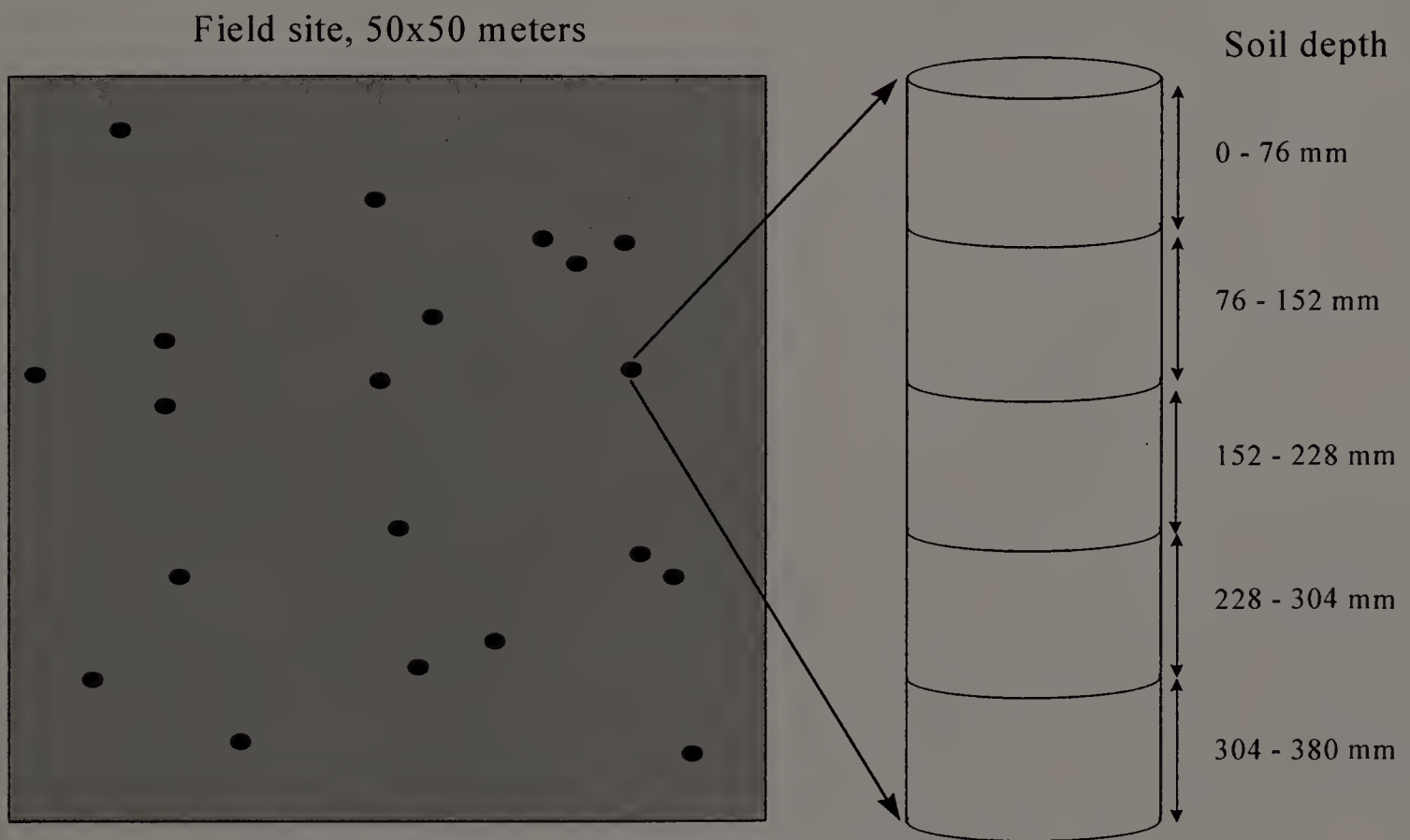


Figure 16: Sampling method. The random sampling pattern is shown on the left and the way individual samples were subdivided is shown to the right. The figure is not to scale.

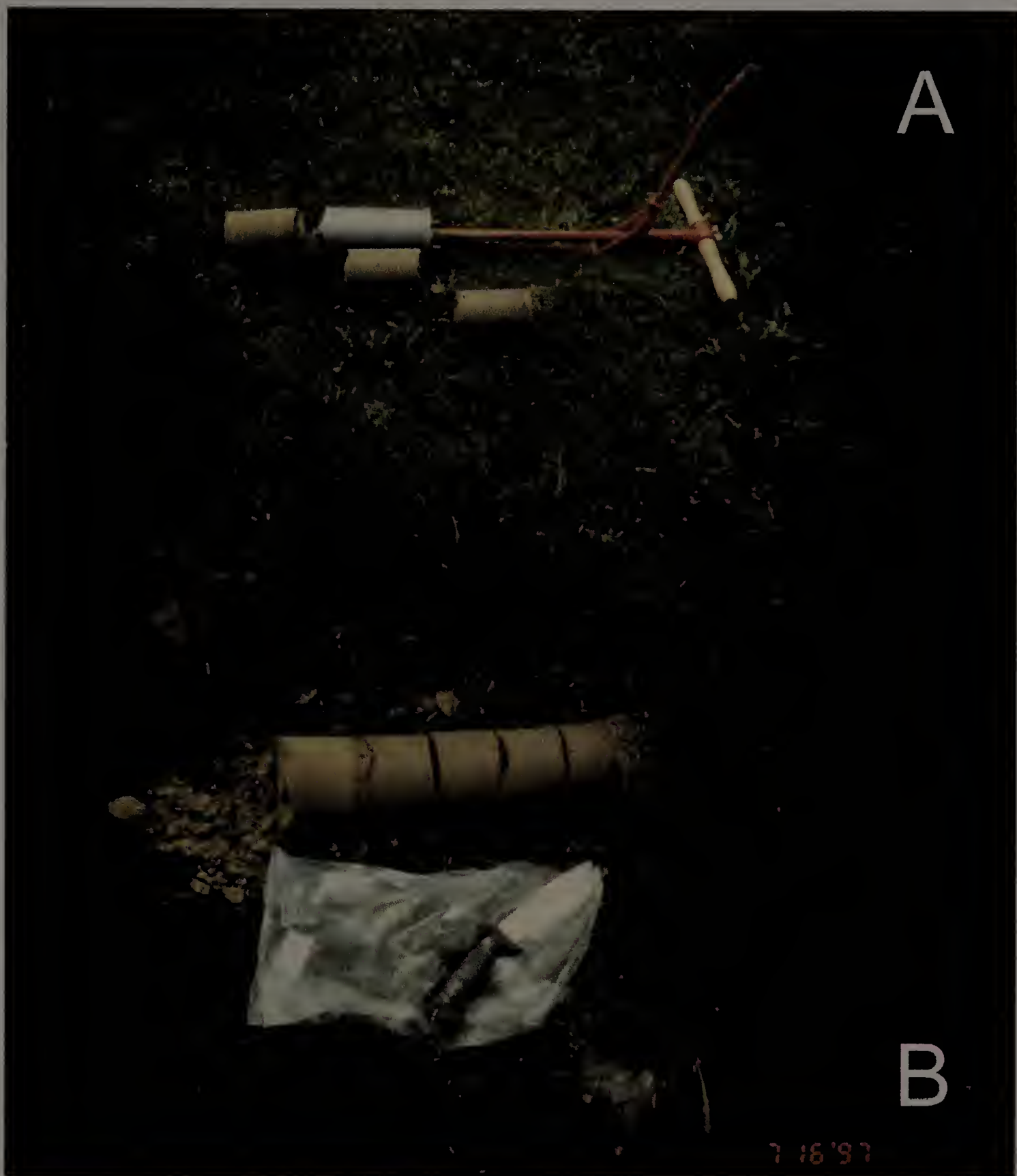


Figure 17: Sampling method. A: cup cutter with three cores. B: cores cut into individual depth increments and ready for bagging.

Samples were prepared for inspection by placing them in a 10°C commercial refrigerator for 7 to 10 days. Then each soil core was sifted under a constant stream of tap water through a 1.5 mm mesh sieve. For 0-76 mm cores the grass and roots were sifted first and discarded, then the remaining soil was added to the sieve. All other cores



were sifted in one step (Figure 18). The contents of the sieve were transferred to clear plastic containers (Figure 19), and turfgrass ants were counted and recorded. This process allowed us to obtain a very accurate count of adults and pupae present in the cores. Eggs were never counted, and while numerous larvae were recovered throughout the study, many larvae were too small or delicate to survive the sifting process.



Figure 18: Extraction of ants from soil core. A 0-76 mm core being prepared for sifting. Initially the grass and root mass was sifted to remove all ants, then the remaining soil was sifted. All sifting was done under a constant stream of tap water.

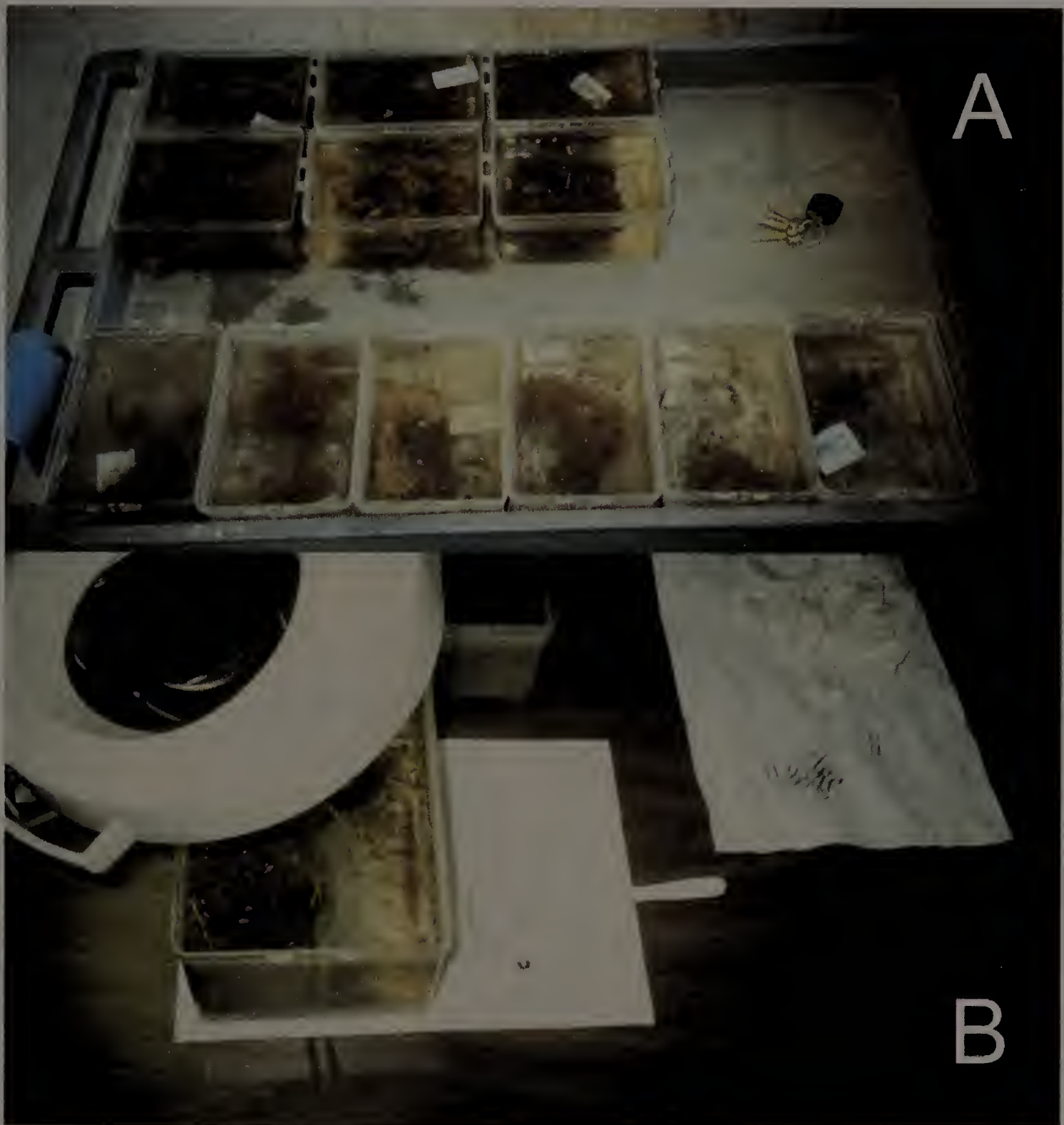


Figure 19: Sample processing. A: 12 samples ready to be counted after being sifted and rinsed in tap water. B: a sample being counted, a white plastic spoon was used to remove ants from the water surface as they were counted.

### Results

The mean number of ants for each of the twenty soil cores taken on a given date (and depth) was determined and a 95% confidence interval estimate was calculated for the mean of all 5 depth strata. These data are presented as bar graphs of the means by

depth and sample date (e.g., Figure 20), and as proportions (percent of the total at each depth) (e.g., Figure 21). Figure 20 also has the overall mean for the entire study (all depths, 0-380 mm) plotted as a band running horizontally behind the bars. The width of this band represents the limits of the 95% confidence interval estimate for this mean.

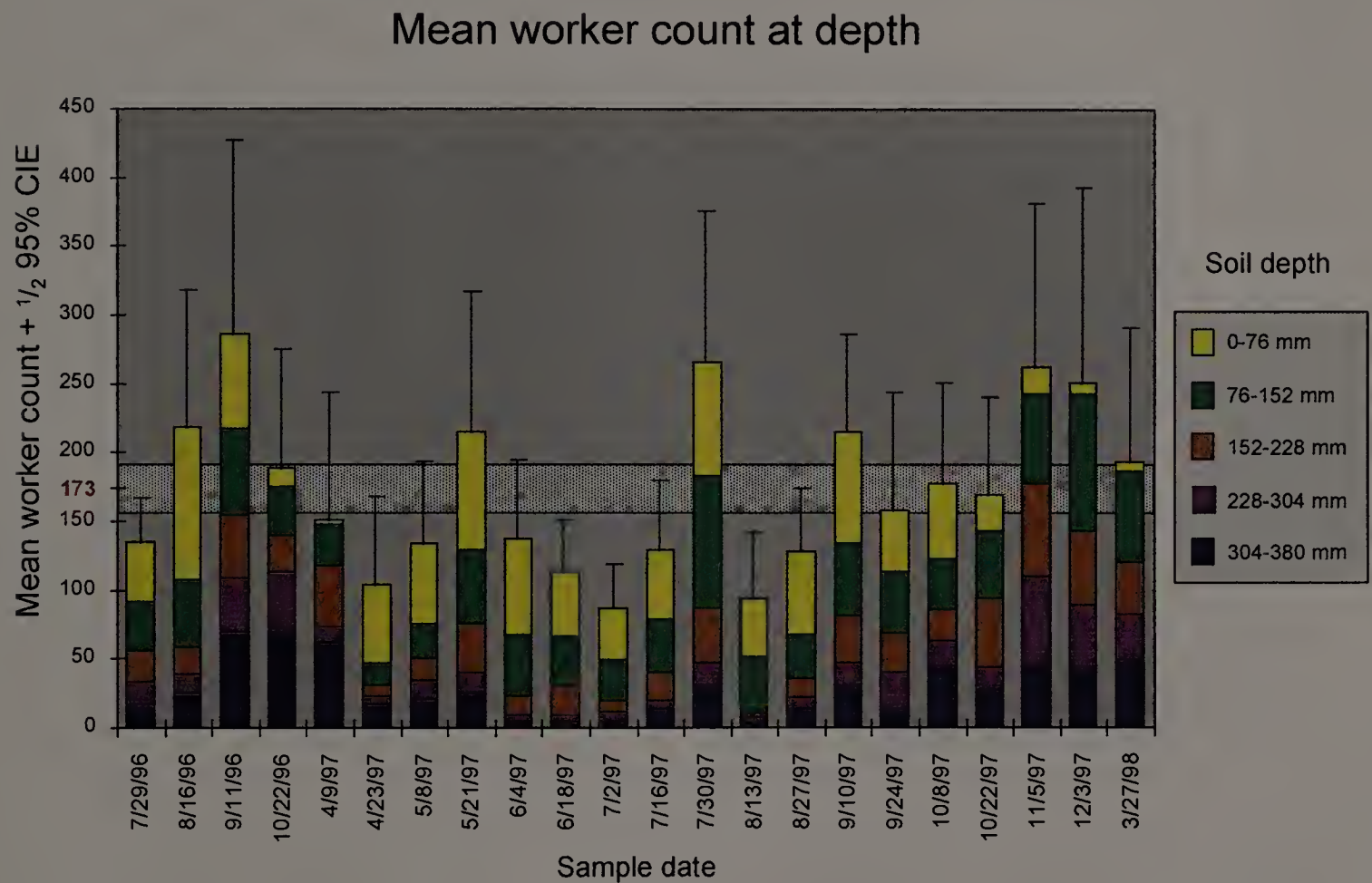


Figure 20: Mean worker ant counts by sample date. Error bars represent the upper limits of the 95% confidence interval estimate for the mean of all depths for a given date. The stippled area behind the bars represents the 95% confidence band for the overall mean of 173, which is also shown.

## Mean worker proportion at depth

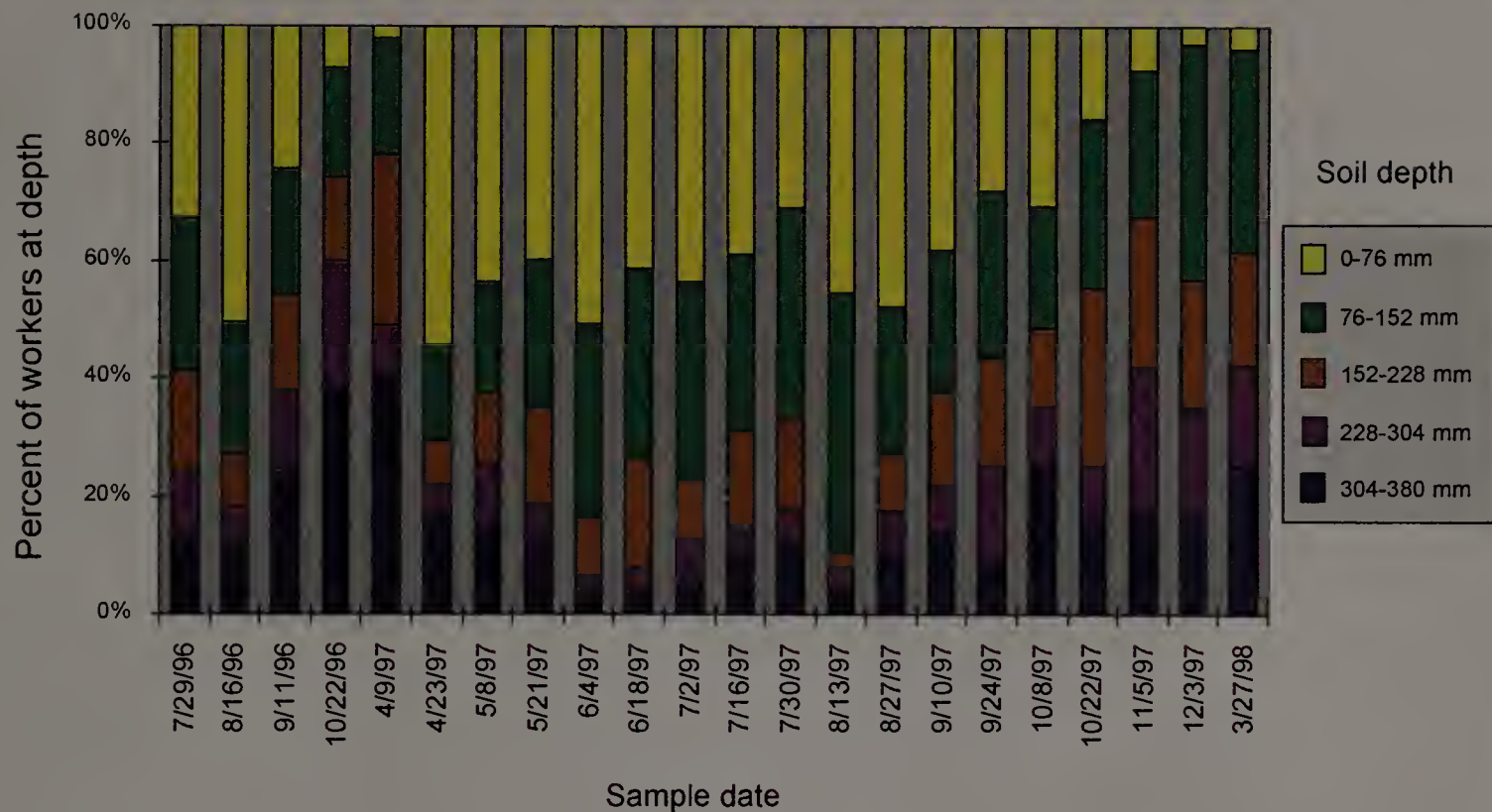


Figure 21: Data from Figure 20 represented as a proportion. Each column is divided into the five depth strata and the percent of total at each depth is represented

Larvae of *Lasius neoniger* are small and soft bodied and as such were not counted accurately by these methods. The extraction procedure used underestimated larval populations and undoubtedly missed many small larvae. Nevertheless, these data are included to show that larvae were found in at least some cores on every sample date except one. These data are presented as both counts (Figure 22) and proportions (Figure 23). However, given the high variance and low counts these graphs are essentially meaningless outside of presence/absence, and that is all that is intended to be conveyed by their inclusion.

### Mean larva count at depth

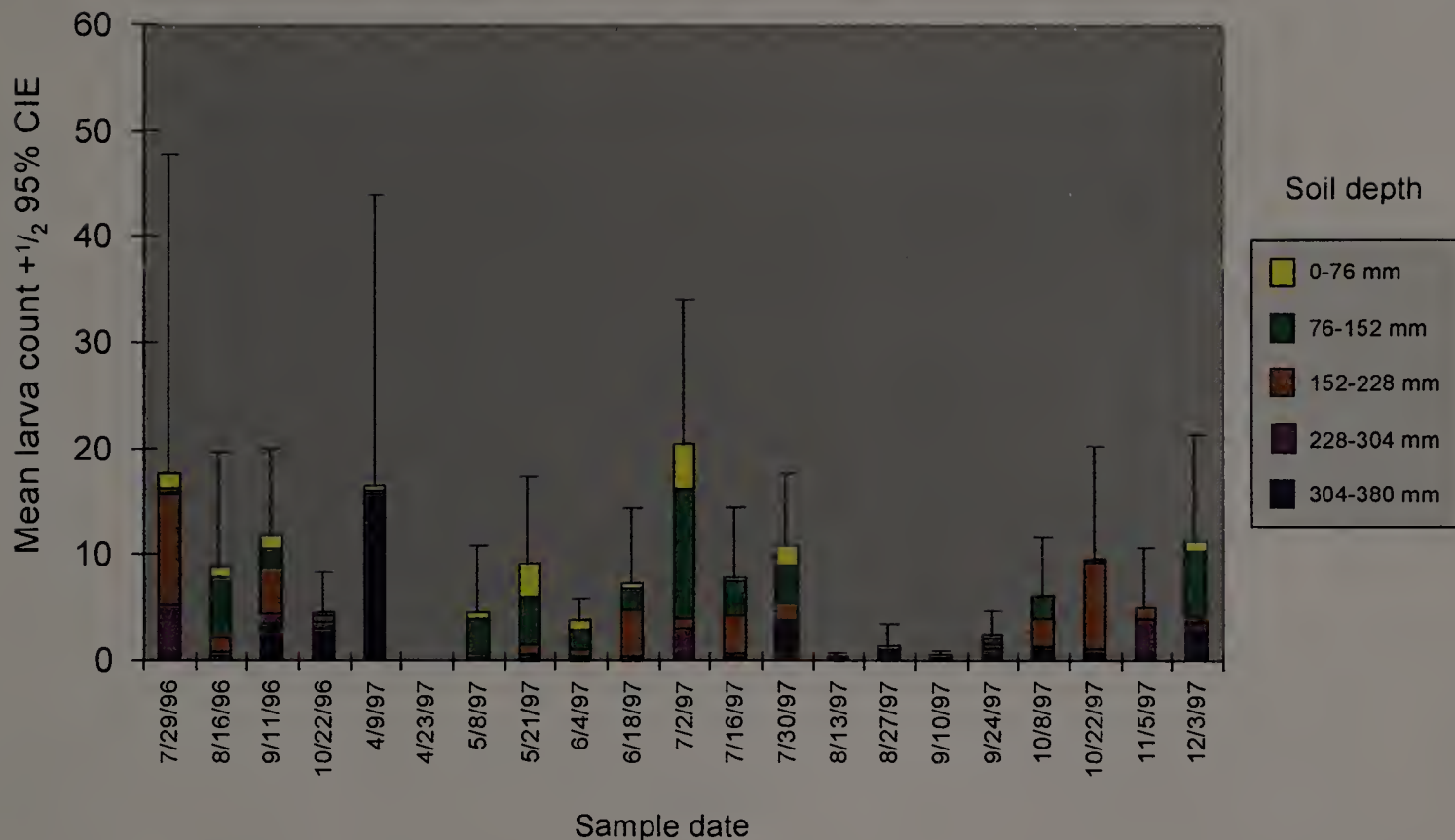


Figure 22: Mean larval ant count by sample date. Error bars represent the upper limits of the 95% confidence interval estimate for the mean.

### Mean larva proportion at depth

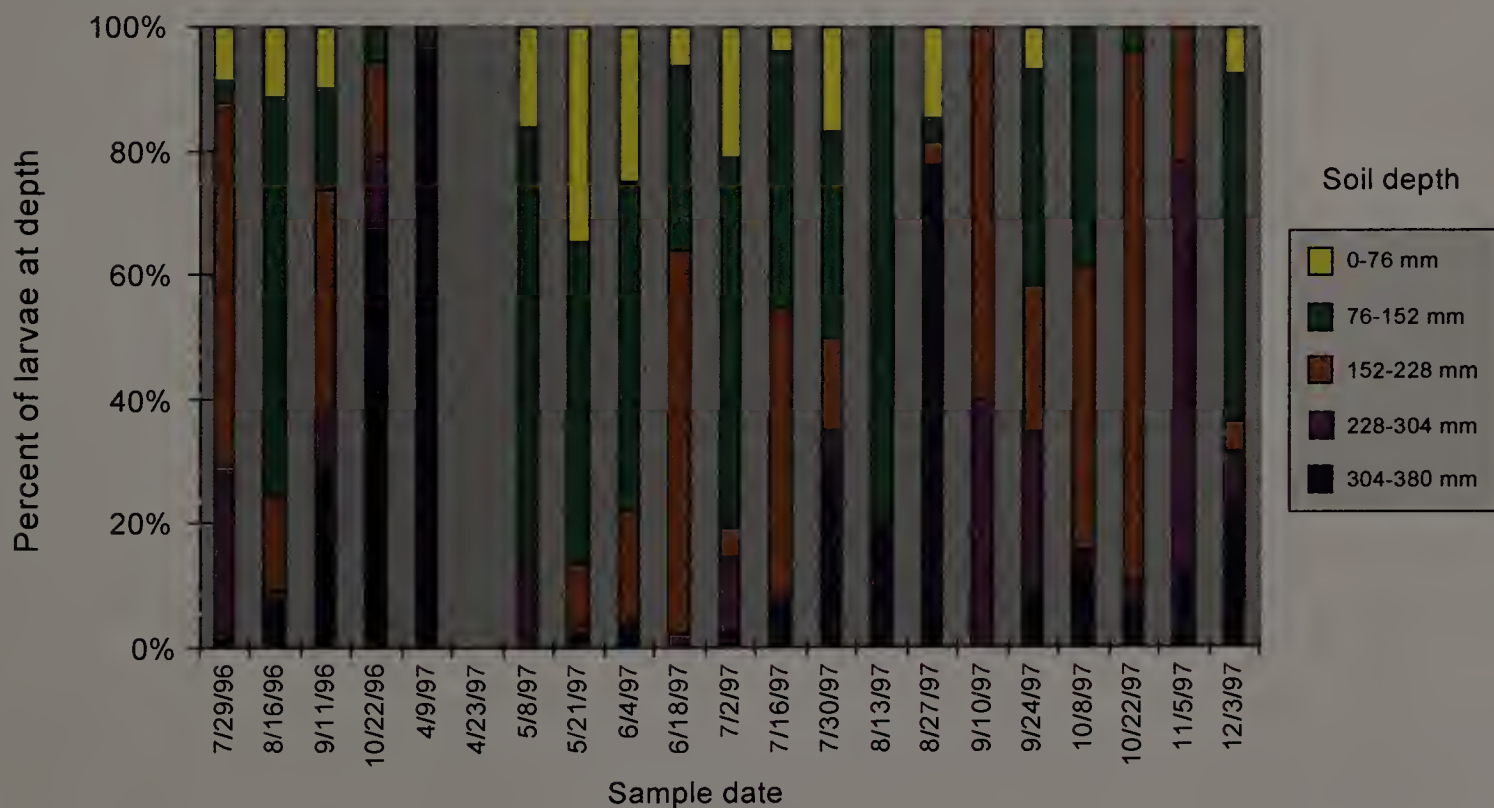


Figure 23: Data from Figure 22 represented as a proportion. Each column is divided into the five depth strata as above but the proportion of ants is represented rather than the actual count.

The pupae of *Lasius neoniger* are wrapped in silken cocoons and separated very well during the sifting process. They first appeared in mid June, increased steadily until the end of July, and then abruptly disappeared (Figures 24 and 25). A Student's t-test was used to compare the mean from the 7/30/97 sample date with the mean from the 8/13/97 sample date, and this test showed that these means were significantly different ( $p < 0.001$ ). This seems to indicate that a discrete cohort of workers develops each year while the colony is at its peak of foraging productivity. This was an unexpected pattern given the apparent year-round presence of larvae. Note, however, that the scarcity of larvae as a result of the extraction process makes it virtually impossible to determine the development pattern of the larvae.

### Mean pupa count at depth

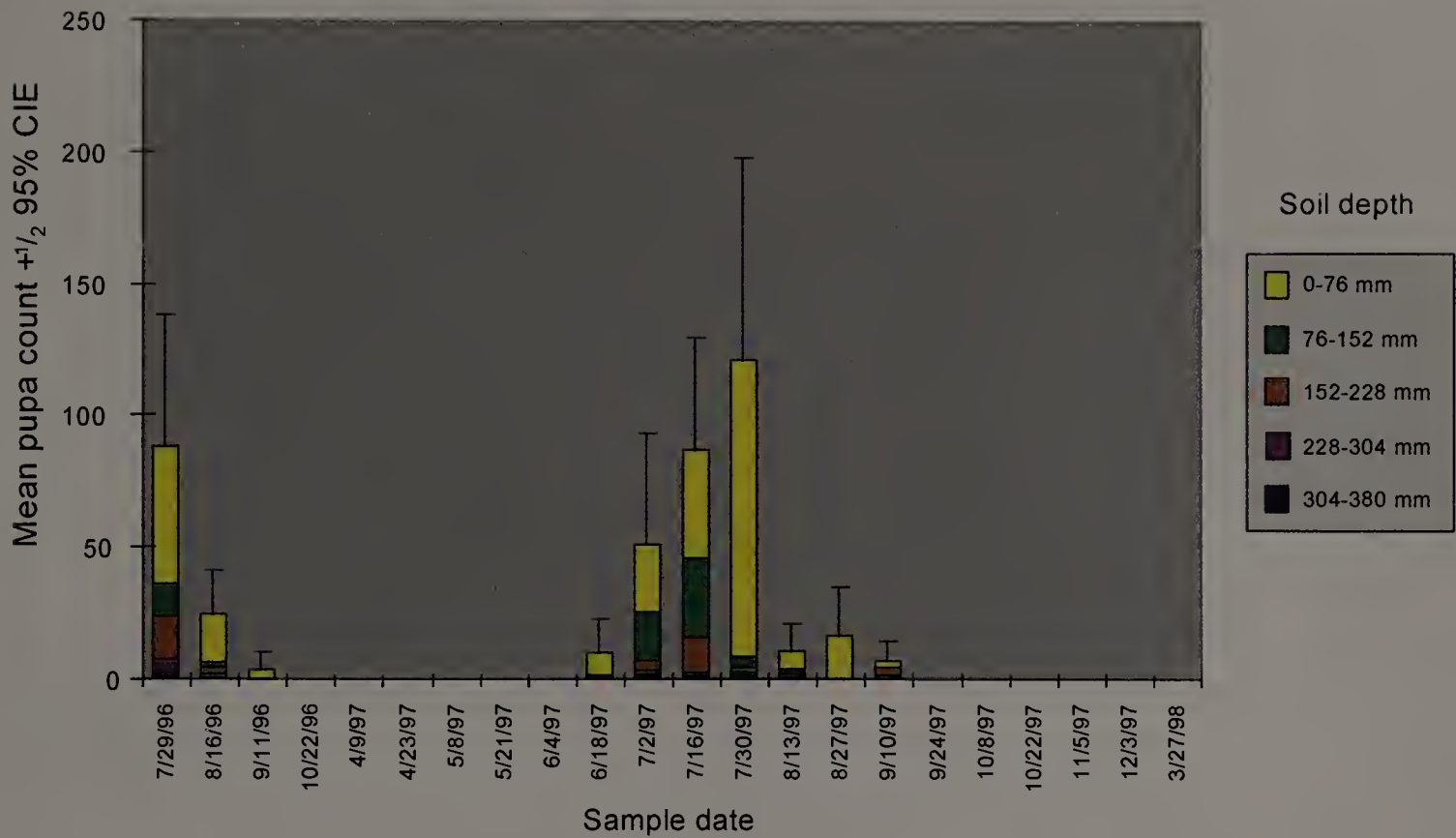


Figure 24: Mean pupal ant count by sample date. Error bars represent the upper limits of the 95% confidence interval estimate for the mean.

### Mean pupa proportion at depth

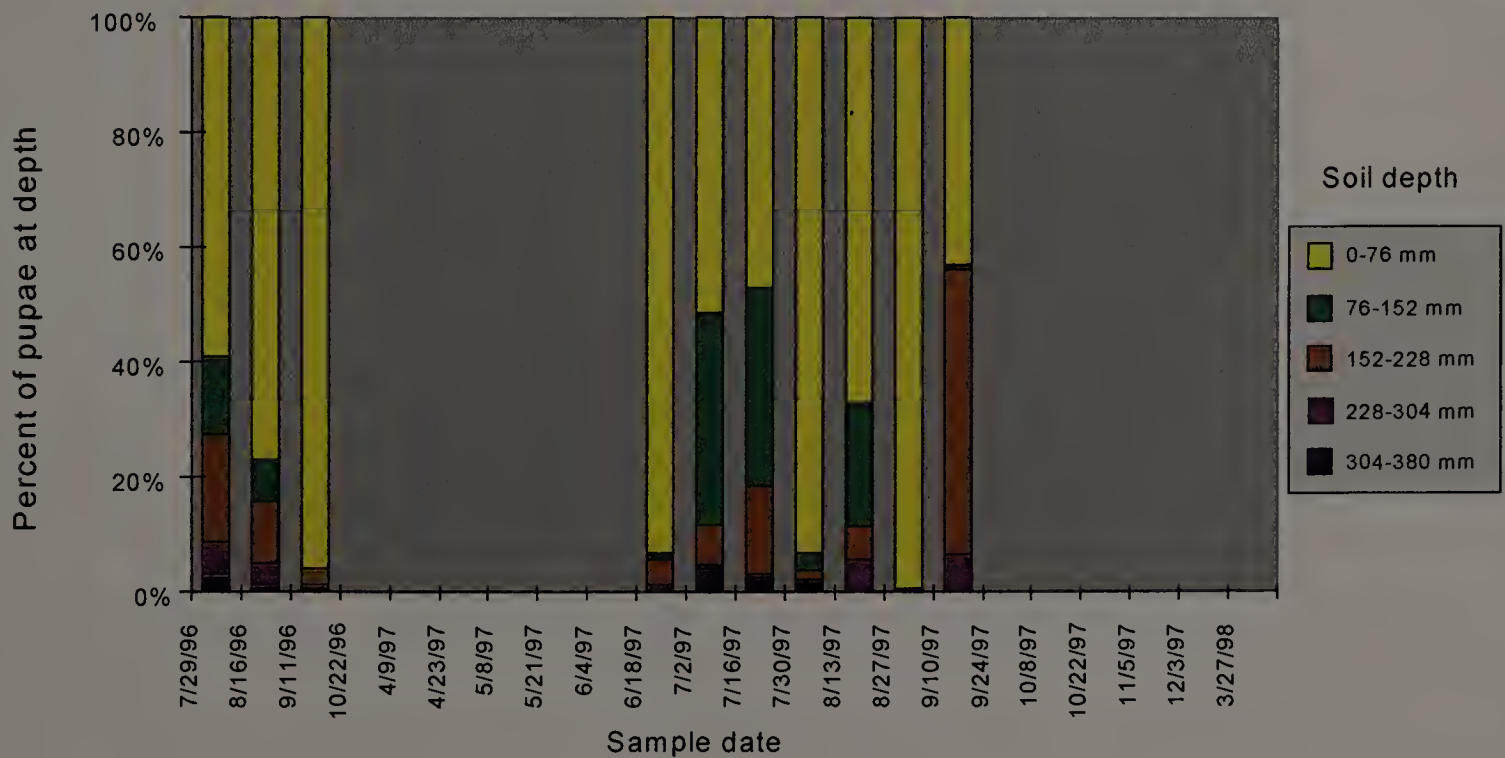


Figure 25: Data from Figure 24 represented as a proportion. Each column is divided into the five depth strata as above but the proportion of ants is represented rather than the actual count.

Winged male ants began to appear in mid July and then were essentially gone by early September (Figures 26 and 27). While sampling in 1996 did not begin until late July, the same phenomenon was observed both years. Due to low counts and high variance, analysis of these data lacks statistical power, but the correspondence between the presence of males and the timing of the nuptial flight in late August/early September is readily apparent. In 1996 there was a significant decrease in alate males between the August 16<sup>th</sup> and the September 11<sup>th</sup> samples, and the nuptial flight was observed that year on August 30<sup>th</sup> and 31<sup>st</sup>. The data are presented as counts and proportions in Figures 26 and 27.



### Mean alate male count at depth

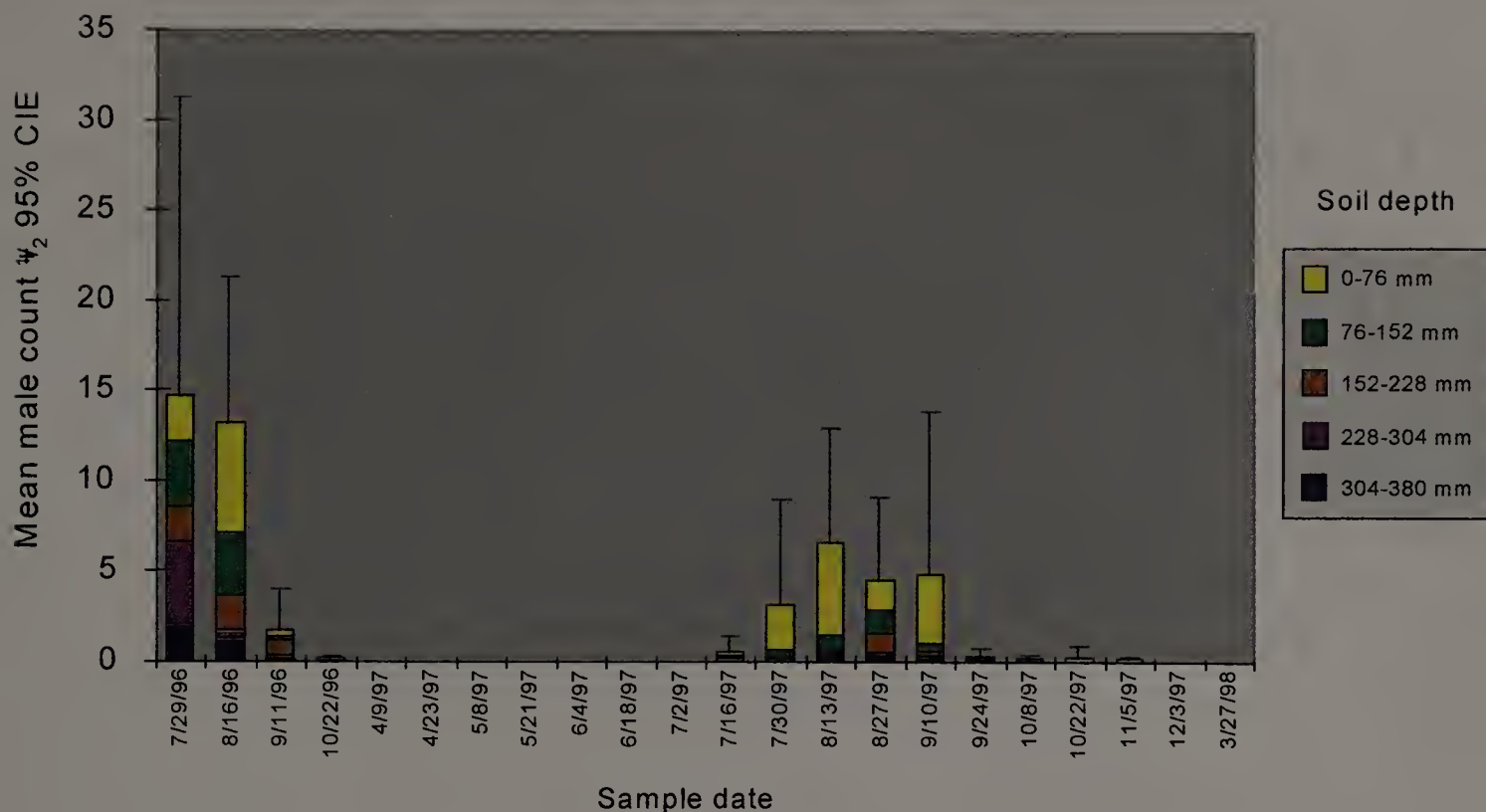


Figure 26: Mean male ant count by sample date. Error bars represent the upper limits of the 95% confidence interval estimate for the mean.

### Mean alate male proportion at depth

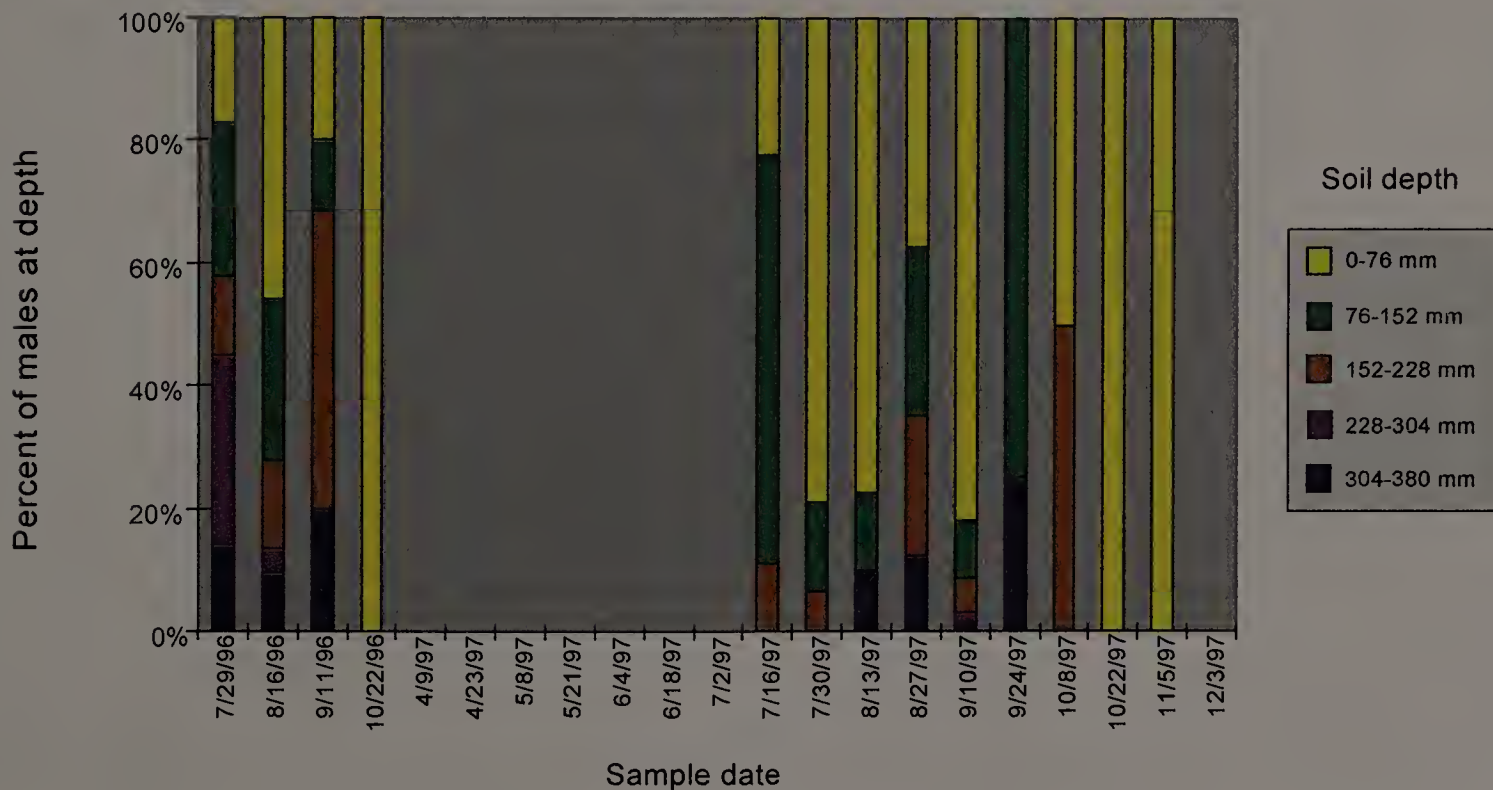


Figure 27: Data from Figure 26 represented as a proportion. Each column is divided into the five depth strata as above but the proportion of ants is represented rather than the actual count.

The data presented in Figures 20-27 illustrate why Wilson (1955) called *Lasius neoniger* the most conspicuous ant in North America. By far the most common life stage encountered in the soil cores was the adult female worker ants. The count per core ranged from 0-882, with 76% of soil cores containing worker ants. Of the 2200 soil cores processed, just under 50% contained at least 10 worker ants, and the overall mean was 35 ants per core. The average number of ant workers per sample (0-380 mm of soil) across the whole study is  $173 \pm 18$ . Each core encompassed  $0.0929 \text{ m}^2$  of surface area; thus, it can be determined that the population was 189 million ants per hectare, or 77 million ants per acre, in just the top 0.38 meters of soil on the study site. Since Wang et al. (1995b) confirmed that turfgrass ant nests reach depths in excess of 1 meter, this population estimate is conservative. Given this population density, it is not surprising that these ants are becoming pests on fine turf.

### Discussion

The population estimate was based on the data presented in Figure 20. The density of workers fluctuated somewhat over the course of the year but overall remained stable around the study-long mean of 173 ants per sample. There were three dates on which worker density was significantly lower than the overall mean; 18 June 97, 02 July 97 and 13 August 97. There does not appear to be any correlation between these dates and temperature and rainfall data as recorded by the weather data logger at the turf research facility. The study site was also irrigated twice a week over the course of the study.

An interesting aspect of these data can be seen more clearly in Figure 21. Here it can be seen that over the winter of 1996-97 about 80% of the ant workers present were found at soil depths greater than 152 mm. (Samples could not be taken during January and February due to the difficulty of taking cores from the frozen soil). Sometime between the samples taken on 9 April 97 and 23 April 97 most of the workers moved to the surface, apparently to begin foraging. For the duration of the summer, more than 80% of the workers were found in the top 152 mm of soil. Then in September the population began to move back into the soil. The winter 1997-98 samples look much as those of the previous winter, with at least 60% of workers below 152 mm. Many soil-dwelling insects overwinter deep in the soil, often below the frost line, and the ants appear to be similarly adapted.

The importance of these observations lies in the timing of pesticide applications used to control turfgrass ants; if materials are applied in early to mid-April, the ants could be prevented from initiating spring foraging activity, a precursor to the damage they ultimately cause, specifically nest expansion and creation of new nest entrances. Some pesticide tests which included different timing of application in the treatments have shown this to be true: applications made just as surface activity becomes apparent seem to control ants for a longer period of time than those that are made earlier or later (Vittum, unpublished data).

Figures 22 and 23 are included to show that ant larvae were recovered from samples throughout the year, including late winter. Nothing else can be inferred from these data because many larvae did not survive the sample processing. The numbers shown undoubtedly represent gross underestimations of larval frequency, but do

document the presence of larvae throughout the year. These data indicate that some ants overwinter as larvae, albeit deep in the soil profile.

The data for pupae, presented in Figures 24 and 25, are very interesting. Worker development seems to be coordinated and emergence from puparia seems to occur simultaneously for the whole year's cohort of new workers. Pupae were never seen before mid-June and then they increased in number throughout the month of July. Some time in the first two weeks of August, the new workers appear to have emerged en masse; there was a significant ( $p < 0.001$ , student's t-test) decrease in pupal counts between the 30 July 97 and the 13 August 97 samples. Interestingly there is no corresponding increase in worker counts as might be expected, in fact the 8/13/97 worker sample was one of the three that were significantly less than the overall mean. It is possible that this is a result of newly emerged workers moving deeper into the soil but this will require further investigation. The pupae were located primarily within the top 152 mm of soil (Figure 25) where they would be most likely to benefit from the warmer temperatures induced by solar radiation.

Finally, Figures 26 and 27 show the development of alate males in preparation for the yearly nuptial flight. Though the variance was high and the counts comparatively low, it can be seen that the number of male ants increases near the surface (i.e. 0-152 mm) in late summer, but alates are no longer recovered from samples collected after mid-September. In 1997, the nuptial flight at the South Deerfield study site was observed to occur in the first week of September, while in 1996 the nuptial flight occurred during the last week of August. These flights were observed to be occurring

elsewhere and a trip was taken to the South Deerfield study site to confirm that the alate ants were emerging there as well. In both 1996 and 1997 this was the case.

All of these data are valuable because they enable turf management professionals to plan their control strategies with the location of the target pest in mind. Without this kind of knowledge, pesticide application or other management strategies are often ill-timed and unnecessary, possibly resulting in overuse of pesticides and the resultant potential for harmful environmental impacts.

## CHAPTER IV

### RESPONSE OF TURFGRASS ANTS TO INSECTICIDE APPLICATION

#### Introduction

*Lasius neoniger* has proven very difficult for turf managers to manage. The ants are subject to attack by a few potential biocontrol agents such as parasitic chalcidoid wasps (Ayre 1962, Heraty 1985, Johnson 1988) and entomopathogenic fungi (Wheeler 1910), but none of these agents has ever proven effective on a large scale. Chemical control methods have also fallen short of efficiently controlling turfgrass ants. Though studies have shown population or mound suppression for up to several weeks (Power et al. 1992, Sloderbeck and Green 1983, 1984, Swier 1996, Swier and Rollins 1996, Vittum, unpublished data), the cost of this control often is not economically feasible, so ants have remained a serious golf course problem in the Northeast.

The reasons for the ants' lack of sensitivity to chemical control are not completely understood, but avoidance is one possible mechanism for such resistance. Over millions of years, all ants have evolved a way of life that is highly dependent on the use of chemical compounds. These compounds function in defense, recruitment to food sources, colony hygiene, colony recognition, and probably in many as yet undescribed ways (Blum and Brand 1972, Hölldobler and Wilson 1990, Wilson 1971). Olfactory nestmate recognition has been suggested as an important factor in the evolution of social behavior in insects (Blum 1987). *Lasius neoniger* workers have been shown to use several pheromones to recruit other workers to a discovered food source (Traniello, 1983). Because of this overarching reliance on chemicals for

communication, it is reasonable to speculate that ants might be able to detect a range of other chemical compounds.

Using the sampling technique described in Chapter II, the behavioral response to surface insecticide application by *L. neoniger* was investigated. The objective of this study was to determine whether ants respond to a surface application of an insecticide by moving deeper into the soil profile to avoid contact with the chemical.

### Methods

This was a preliminary study and as such was not truly replicated. The study was conducted on mowed turfgrass plots located at the University of Massachusetts' turf research facility in South Deerfield, Massachusetts. The plot was maintained like a typical lawn (Kentucky bluegrass/perennial ryegrass blend), established in 1989, and mowed weekly at a height of 5 cm. The plot was irrigated twice a week. Each plot was 9 meters square and was subdivided into nine 3 by 3 meter subplots in order to help eliminate heterogeneity in the pesticide applications. There were three plots, one untreated control and two that were treated with an insecticide. Plots were separated by 3 meters of untreated turf. In 1997 the materials tested were chlorpyrifos (Dursban Pro) and cyfluthrin (Tempo 20 WP). The application rates were 0.908 kg of active ingredient per acre for Dursban Pro and 0.158 kg active ingredient per hectare for Tempo 20 WP. The insecticide was pre-measured in the laboratory and applied in 3 liters of water using an 8 liter watering can, for each of the nine subplots. Standard galvanized steel garden watering cans were used for soluble materials, granular materials were applied using 0.5 liter shaker jars. Irrigation equivalent to 7 mm of rainfall was then applied to all plots.

Using the technique described in Chapter III, 15 samples per plot at 75 mm increments to a depth of 380 mm were taken prior to pesticide application. Subsequent samples were then taken 1, 3, 7 and 14 days after application. Samples were always taken within 1.5 hours of 12:00 (clock) noon. Samples were bagged, tagged, frozen, and subsequently sifted in water as described in Chapter III. Counts of worker ants were recorded and mean values calculated. Only data from worker ants are presented here because the larval and pupal stages are incapable of moving independently and must be carried by workers. Since this investigation was focused on the ability of ants to respond to chemical applications, only the mobile stage was sampled.

In 1998 the materials tested were Fipronil 0.1G at 0.055 kg AI per hectare and Deltagard at 0.158 kg AI per hectare. The sampling method was modified slightly to increase statistical power. Instead of 15 samples per plot, 20 samples were taken, but only 4 depth strata were sampled, 0-76 mm, 76-152 mm, 152-228 mm, and 228-304 mm. These samples were then processed as above.

### Results and Discussion

The results of this study are presented below in Figures 26 (1997) and 27 (1998). 95% confidence interval estimates were calculated for each day's mean count. Heavy rain occurred on the day after application in 1997 and this precluded taking a full set of samples because the sampling process was impossible to carry out in rainfall. On this date, only the control and Dursban Pro plots were sampled.



### Pesticide avoidance test, 1997

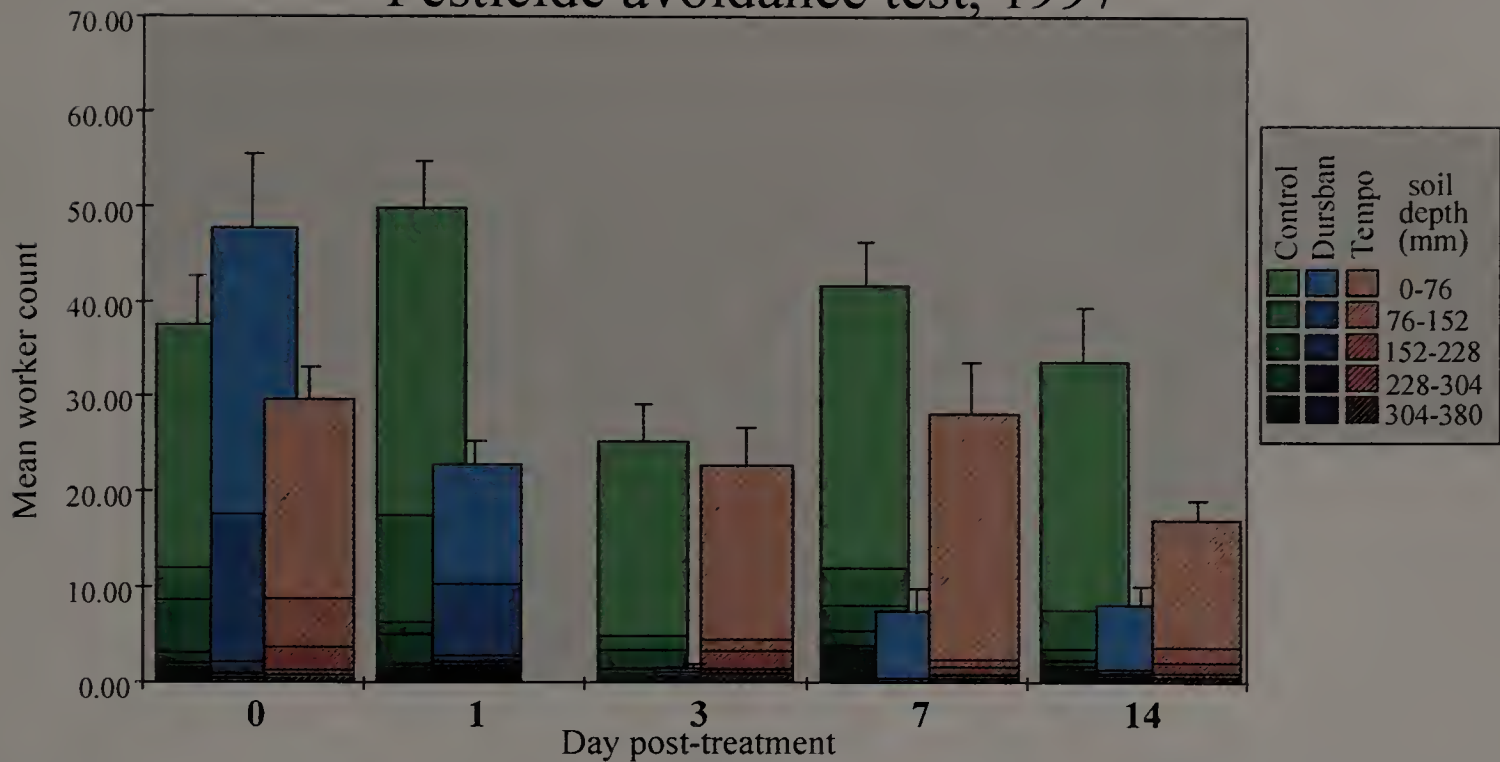


Figure 28. Mean worker counts versus days post-treatment for untreated turf and for the two materials tested in 1997. Missing data for Tempo 1 day post-treatment was due to rain making sampling impossible. Error bars represent the upper limit of the 95% confidence interval estimate for the mean.

### Pesticide avoidance test, 1998

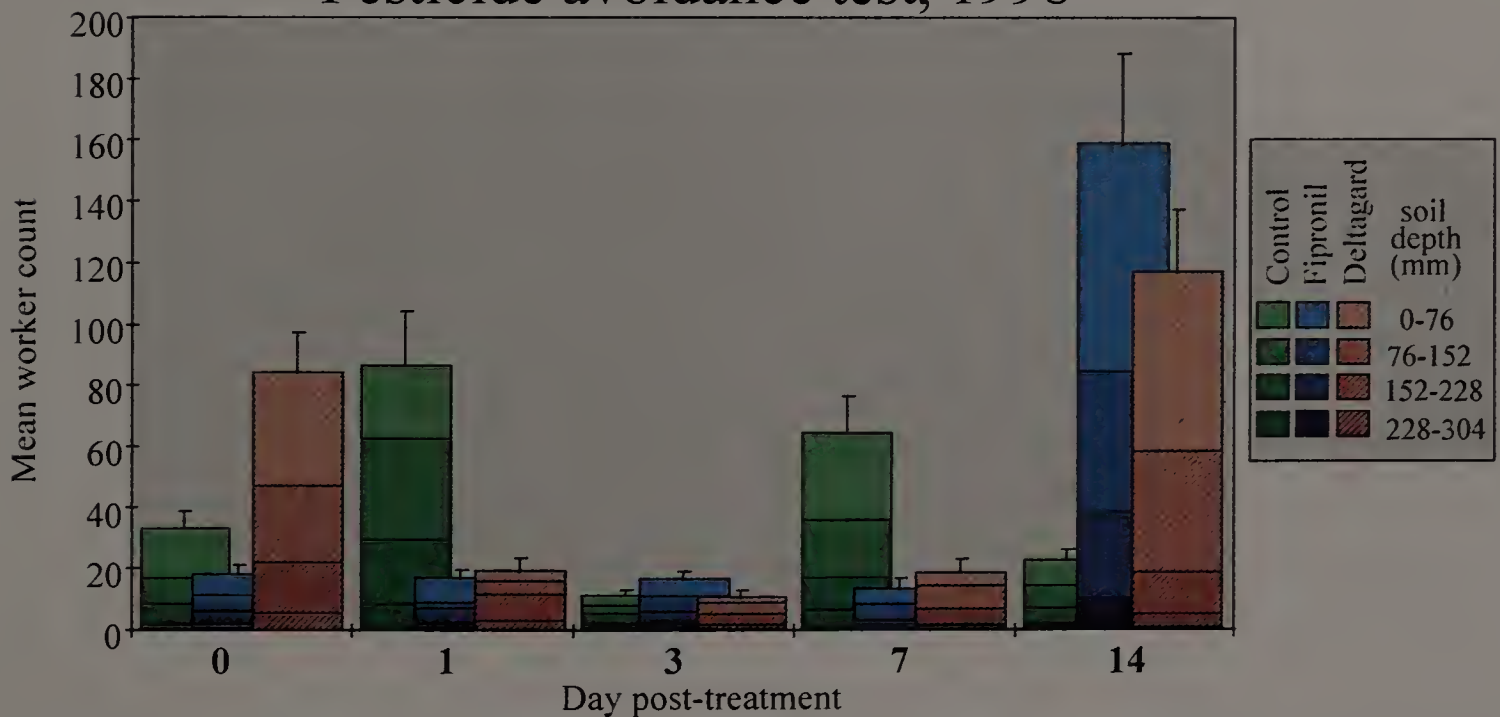


Figure 29. Mean worker counts versus days post-treatment for untreated turf and for the two materials tested in 1998. Error bars represent the upper limit of the 95% confidence interval estimate for the mean.

The 1997 test result could be interpreted as evidence of avoidance behavior in the plot treated with Dursban Pro, although other possible explanations exist (such as mortality that occurs after the ants leave the plot in the course of normal foraging). Figure 28 shows a significant decrease on day one (based on the lack of overlap between the 95% confidence intervals that are plotted as error bars), a further decrease on day three, and the ants remained significantly reduced in number for the remainder of the study. It is important to note that the sampling method used could not distinguish between ants killed by insecticide and ants killed by freezing (of the samples). Therefore the reductions observed in this experiment must have been a result of ants leaving the treated plot. It is also interesting to note that as ants began to return to the treated plots on Days 7 and 14, they were showing up in the shallowest depth category, suggesting that they were returning over the land surface rather than from deeper in the soil. The Tempo plot showed significant reduction on days 7 and 14, indicating that avoidance was taking place but with a longer response time than that observed for Dursban Pro. Again, most ants recovered in the soil cores appeared in the shallowest depth category.

In 1998 the data were difficult to interpret due to highly fluctuating population numbers across all plots. The pattern that seems to emerge is one of significant avoidance of both materials on day 1, abandonment of the untreated control plot by day 3, repopulation of the control plot by day 7, and a resurgence in the treated plots after two weeks.

These chemical avoidance tests were preliminary in nature and as such, the treatments were not replicated. The data, however, indicate that further study is

warranted. A larger test with replicated treatments should be conducted with these materials (which are currently labeled or used for ant control) and with other materials being considered for applications targeted against turfgrass ants. While logistical constraints are daunting, such tests would yield valuable information about behavioral responses of turfgrass ants to insecticide applications and enable turf managers to use materials more wisely – or refrain from using them when ant behavior is likely to lead to avoidance.

APPENDIX

TAXA FOUND IN PITFALL TRAPS.

This appendix is included in order to give some information about the various taxa that were collected in the pitfall traps described in Chapter 2. Overall, more than 18000 arthropods were collected and identified. They are arranged in Table 2 by decreasing order of overall abundance.

Table 2. Brief notes on taxa found in pitfall traps.

Taxon	count	% of total	Information
<i>Lasius neoniger</i>	6654	36.8	Turfgrass ants
Staphylinidae	5667	31.3	Rove beetles, roughly 5 predatory species present
<i>Solenopsis molesta</i>	1571	8.7	Thief ants, closely related to fire ants.
Diptera	1340	7.4	Flies, roughly 20 species present
Carabidae	632	3.5	Ground beetles, roughly 5 predatory species present
Lathridiidae	377	2.1	Minute brown scavenger beetles, mold and detritus feeders
Aranaea	295	1.6	Spiders, roughly 10 species present, predators
Cryptophagidae	243	1.3	Silken fungus beetles, scavengers of mold and detritus
Curculionidae	189	1.0	Weevils, 3 or 4 species present, mostly bluegrass billbug
Diplopoda	167	0.9	Millipedes, detritus feeders
Histeridae	132	0.7	Hister beetles, predators similar to ground beetles
Myrmecinae	125	0.7	Myrmecine ants, (excluding <i>Solenopsis molesta</i> )
Scarabaeid	89	0.5	Scarab beetles, roughly 8 species present
Elateridae	83	0.5	Click beetles, about 4 species
Nitidulidae	80	0.4	Sap beetles, about 4 species
Dermaptera	79	0.4	Earwigs, detritus feeders
Eucoilidae	60	0.3	Parasitic wasps
Scelionidae	44	0.2	Parasitic wasps
Tenebrionidae	42	0.2	Darkling beetles, several species
Chrysomelidae	38	0.2	Leaf beetles, mostly flea beetles (subfamily Alticinae)
Aphidae	29	0.2	Aphids, several species
<i>Brachymyrmex depilis</i>	21	0.1	Small formicine ants closely related to turfgrass ants
Lygaeidae	19	0.1	Seed bugs, plant feeders, one species
Ptiliidae	14	0.1	Feather-winged beetles, mold feeders

Continued next page

Table 2. Continued

Taxon	count	% of total	Information
Lepidoptera	12	0.1	Butterflies or moths, mainly Noctuidae (cutworms)
Diapriidae	10	0.1	Parasitic wasps
Pteromalidae	8	0.0	Parasitic wasps
Camponotus	7	0.0	Carpenter ants
Rhizophagidae	6	0.0	Rhizophagid beetles, detritus feeders, one species
Leiodidae	6	0.0	Round fungus beetles, mold feeders, rare
Apidae	5	0.0	Bees, mainly bumble bees, some honey bees
Chalcidae	5	0.0	Parasitic wasps
Ceraphronidae	5	0.0	Parasitic wasps
Scolytidae	5	0.0	Bark-and-ambrosia beetles, wood boring beetles, one species
Corylophidae	5	0.0	Minute fungus beetles, rotting plant material, mold feeders
Mycetophagidae	5	0.0	Hairy fungus beetles, fungus feeders
Ichneumonidae	4	0.0	Parasitic wasps
Protoctrupidae	3	0.0	Parasitic wasps
Psocoptera	3	0.0	Bark lice, lichen feeders
Anthicidae	3	0.0	Antlike flower beetles, scavengers
Lasius umbratus	3	0.0	A close relative of the turfgrass ant, woodland species
Dermeestidae	2	0.0	Dermeestid beetles, feed on dry decaying matter
Ponera pennsylvanica	2	0.0	The only ponerine ant in the northeast, small woodland species
Saldidae	2	0.0	Velvet shore bugs
Cicadellidae	2	0.0	Leaf hoppers
Heteroceridae	1	0.0	Variegated mud-loving beetles, scavengers, stream dwellers
Formica sp.	1	0.0	Wood ants, surprising that they weren't more common in traps
Braconidae	1	0.0	Parasitic wasps
Cynipidae	1	0.0	Parasitic wasps
Chilopoda	1	0.0	Centipede, predatory arthropod
Blattaria	1	0.0	Roach, detritus feeders
Cercopidae	1	0.0	Frog-hoppers, plant feeders
Nabidae	1	0.0	Nabid bugs, predators
Buprestidae	1	0.0	Buprestid beetles, wood borers
Dryinidae	1	0.0	Parasitic wasps
Eurytomidae	1	0.0	Parasitic wasps
Lampyridae	1	0.0	Lightningbugs or fireflies, actually beetles
Pompilidae	1	0.0	Spider wasp, parasitic on Aranaea

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