THE ECOLOGY AND THE BIOLOGICAL CONTROL OF THE ANNUAL BLUEGRASS WEEVIL, *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE) USING ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: STEINERNEMATIDAE AND HETERORHABDITIDAE)

by

BENJAMIN ALEXANDER M^{c}GRAW

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ABSTRACT OF THE DISSERTATION

THE ECOLOGY AND THE BIOLOGICAL CONTROL OF THE ANNUAL BLUEGRASS WEEVIL, *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE) USING ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: STEINERNEMATIDAE AND HETERORHABDITIDAE)

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BENJAMIN ALEXANDER M^{c}GRAW

Dissertation Director:

Dr. Albrecht Koppenhöfer

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is a highly destructive insect pest of fine turfgrass in the northeastern United States and eastern Canadian provinces. I examined the spatial ecology of *L. maculicollis* and assessed the virulence of endemic and released entomopathogenic nematodes to weevil stages to develop ecologically based control programs. Endemic populations of *Steinernema carpocapsae* Weiser and *Heterorhabditis bacteriophora* Poinar infected a range of weevil instars and caused moderate generational mortality. The variability in seasonal abundance of endemic nematode populations and the variability in weevil generational

mortality suggests an inability for reliable pest population regulation. Laboratory bioassays demonstrated that *L. maculicollis* fourth- and fifth-instar larvae were moderately to highly susceptible to nematode infection. Several species of nematodes significantly reduced densities of both instars, although a decrease in susceptibility to nematodes was observed as the insect aged. No difference was observed between the virulence of endemic and commercial nematode strains to any *L. maculicollis* stage tested. Field trials conducted over a three year period demonstrated great variability in the ability of commercial and endemic nematodes applied at standard field concentrations to reduce *L. maculicollis* densities below damage thresholds. Many factors, including nematode concentration, weevil spatial distribution and density, and timing of application are believed to have contributed to the variability in control.

The spatio-temporal distribution of emerging overwintering adult populations, first generation larvae and the distribution of host plants were examined to identify the spatial structure of populations, better target curative controls and develop monitoring programs. Significant aggregations of cumulative adult captures, larvae and their preferred hosts (*Poa annua* L.) were found on fairway edges when the entire width of fairways was sampled. Adult distribution rarely coincided with the following week's spatial pattern, suggesting that adults actively disperse across fairways throughout the oviposition period. Spatial association was detected between adults and larvae, but rarely between either stage and *P. annua*. The findings challenge assumptions of *L. maculicollis* host preference, but suggest a potential for targeting controls. The data were used to develop sequential sampling programs to rapidly assess adult densities and estimate the threat of larval damage.

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CHAPTER ONE

INTRODUCTION

The annual bluegrass weevil, *Listronotus maculicollis* Kirby (formerly *Hyperodes* sp. near *anthracina, - anthracinus*) is the single most destructive insect pest of fine and golf course turfgrass in the northeastern United States and some areas within the provinces of Ontario and Quebec, Canada (Vittum et al. 1999; Simard et al. 2007). Damage from the weevil is especially evident in short-mown, well irrigated turfgrass stands (e.g. fairways, collars, and greens) with high percentages of annual bluegrass (*Poa annua*). The public demands for the highest quality turfgrass has caused turfgrass managers to adopt low thresholds for damage and to rely solely on preventive control strategies for managing *L. maculicollis* populations. Turfgrass managers around the epicenter of the weevil's distribution (the metropolitan New York area, New Jersey and southern Connecticut) may apply six to ten applications of broad spectrum chemical insecticides per year to reduce egg laying in hopes of avoiding larval feeding damage (McNeil et al. 1999; D. Oatis, personal communication). The reliance on preventive controls and low damage thresholds has led to spatial and temporal misuse of chemical insecticides. Multiple applications made each year for successive years has contributed to the development of pyrethroid resistant populations in areas of the weevil's distribution where populations are the densest (Cowles et al. 2008). The reliance on preventive chemicals to manage *L. maculicollis* and low damage thresholds are at direct odds with conducting ecologically sound Integrated Pest Management (IPM).

Furthermore, we lack a fundamental understanding of *L. maculicollis* biology and ecology which inhibits the ability to adopt sustainable pest management tactics.

History and Taxonomic classification of *Listronotus maculicollis*

The history and taxonomic placement of the annual bluegrass weevil is one that is unclear at best. It is not known exactly how long the weevil has been damaging turfgrass in the northeastern United States or whether it is a native or introduced species (however, the former is believed to be true). A review of the literature does not clarify whether *L. maculicollis* as we know it today or related weevils were causing damage to golf course turfgrass beginning in the 1930's (Britton 1932). Some of the ambiguity in the history of the *L. maculicollis* is related to the classification and reorganization of the genera *Hyperodes* and *Listronotus* within the Curculionidae family.

The genus *Listronotus* is classified in the order Coleoptera, superfamily Curculionoidea, family Curculionidae, subfamily Cylindrorhininae. There has been much revision of the genus over the years. O'Brien (1979; 1981) states that the two genera are synonymous and adds 35 species of *Hyperodes* for a total of 62 species of *Listronotus* occurring in the United States and Canada. Morrone (1997) provides an extensive analysis of the New World Listroderina and distinctively separates *Listronotus* and *Hyperodes* based on 53 morphological characters. More recently, Arnett et al. (2002) maintain the synonymous grouping and place 81 species from the United States and Canada in the genus.

In the mid 1960's it became apparent that small weevils were damaging turf on several golf courses in Nassau and Westchester counties in New York state. Cameron

and Johnson (1971) suggest that the insect could have been causing "spring die-out" that had been observed on Long Island golf courses with high percentages of *P. annua* for nearly 40 years. Britton (1932) was the first to report a species of "*Hyperodes*" damaging golf course turf in Farmington, Connecticut. But due to the number of different species classified as *Hyperodes* (at that time) found in the golf course environment (Cameron and Johnson 1971) it is unclear whether or not the weevil causing damage are the same that was identified in 1965 and commonly found damaging turf today.

R. E. Warner (1965) was the first to identify *L. maculicollis* in samples taken in 1957 and 1961 from damaged turf from Long Island, New York and Pennsylvania. She classified the insects as *Hyperodes anthracinus* (Dietz). H. Dietrich examined the same specimens and identified the specimens from Long Island to be *H. maculicollis* (Kirby), a species that is present in all 50 states (Cameron and Johnson 1971). Around the same time, Shread (1970) reports damage to turfgrass in Connecticut by two separate species, *H. maculicollis* and *H. anthrosinous* and becomes the first to refer to the weevils as "annual bluegrass weevil" for its apparent preference for *P. annua*. Warner later revises her classification of Long Island specimens in the Cornell University collection to "represent a species between *H. anthracinus* and *H. maculicollis*," and thus designated the species to be *Hyperodes* sp. near *anthracinus* (Dietz) (Cameron and Johnson 1971). In 1985, C.W. O'Brien places *L. maculicollis* in its current genus *Listronotus*, and designated the weevil as *L. maculicollis* (Vittum et al. 1999). Although this classification has remained for over 20 years, the alternative or common name "*Hyperodes* weevil" still persists and in most areas is even more common than "annual bluegrass weevil" with golf course superintendents.

Currently there is debate on the authority of *L. maculicollis*. Kirby is considered the authority according to several species checklists and university collections, including Cornell University (www.entomology.cornell.edu/CUIC) and the Integrated Taxonomic Information System (www.itis.gov). However, most publications following the reorganization of the genus *Listronotus* have continued to cite Dietz as the authority. Current understanding amongst taxonomists (Arnett et al. 2002) is that Kirby was the first to describe specimens from the genus *Macrops*, later to be regrouped in either *Hyperodes* or *Listronotus* by Jekel in 1865. *Listronotus* (= *Macrops*, =*Hyperodes*) *maculicollis* was one of these specimens. Dietz described many species of *Hyperodes*, including *H. anthracinus* (now *L. anthracinus*), the species that specimens damaging turfgrass on Long Island were thought to be (or at least very closely related to). Thus, when annual bluegrass weevils were classified by Warner to be *Hyperodes* sp. near *anthracinus*, Dietz was properly cited as the authority. However, even after O'Brien's (1979) reclassification of *Hyperodes* to *Listronotus* many authors continue to cite Dietz rather than reverting to Kirby, the authority of *Listronotus* (= *Macrops*, =*Hyperodes*) *maculicollis*.

Seasonal history of *L. maculicollis*

Current belief is that *L. maculicollis* undergoes two to three generations per year around the epicenter of its distribution (metropolitan New York city). In the most southern or northern areas of its distribution, the weevil's phenology may diverge substantially. Though *L. maculicollis'* geographical distribution is not extensive, the life cycle can differ significantly within its range and between years, largely based on the

weather. Northern populations (central Connecticut to southern Vermont) most often will undergo two generations a year, whereas southern populations (central New Jersey to Maryland) typically pass through three generations. In the epicenter of the distribution, three generations per year will occur in summers with above-average temperatures and two generations in colder summers (Vittum 1980; Vittum et al. 1999).

The adult spends the winter months in overwintering sites in leaf litter and tall grasses adjacent to high-valued short-mown turfgrass sites (Diaz 2006). Adults emerge from these sites over several weeks from late March to late April in New Jersey. Plant phenology is commonly used as an environmental indicator for estimating peak densities and timing chemical insecticide applications. Forsythia (*Forsythia* spp.*)* and flowering dogwood (*Cornus* spp.) do not have any direct effect on *L. maculicollis* development but are reliable indicators of adult presence on fairways (Tashiro et al. 1978). Rothwell (2003) determined that adults begin to move from overwintering sites in leaf litter off fairways by walking, rather than flying in search for hosts. Adults can move considerable distances from overwintering sites to the preferred feeding sites of short-mown fairway grasses (Diaz 2006). The effort to lay eggs in shorter turfgrasses appears to have consequences for larval fitness as larvae seem to develop better in shorter turfgrass (Rothwell 2003).

Once on the short mown playing surfaces of the golf course, mated *L. maculicollis* females chew small holes into the stem of the turfgrass plant, through one or two sheaths and deposit cylindrical eggs approximately 0.8 mm in length and 0.25 mm wide. The eggs first appear to be yellow and transparent, turning olive green to black before hatching. Eggs may be laid singly or end to end, in groups of up to six. Female

weevils are capable of laying eggs for several weeks, resting for several days between oviposition events. Cameron and Johnson (1971) found that female *L. maculicollis* lay an average of 11.4 eggs in the laboratory but found upward of 62 oocytes in field collected females. The eggs hatch in 4.6 d at 26.7^{o}C in laboratory growth chambers.

The larval stage consists of five instars (Cameron and Johnson 1971), and time of development varies depending on the environmental conditions, particularly temperature and quality of food sources. Throughout its development, a typical larva can destroy between 12 and 20 stems (Cameron and Johnson 1971). The tunneling of the grass stem by early instar larvae causes yellowing of the plant. Late-instar larvae emerge from the plant into the soil and feed externally upon the crown, which kills the plant. The spatial distribution of damage by the first-generation larvae is often localized around the perimeters of short-mown turf (edges of fairways, collars, edges of tees, etc). If populations go untreated, high densities can damage a greater area of turf and the patches coalesce into large irregular patches several meters into the fairway from the rough border.

Host Plant Use

It was long believed that *L. maculicollis* solely fed upon *P. annua*. *Poa annua* is a highly invasive, bunch type grass weed that is extremely prevalent in temperate regions such as the northeastern United States. It is considered a "winter annual" since it completes it life cycle in a year, producing seed from which it germinates in the fall, and dying in the following summer. However, constant irrigation coupled with low mowing heights typically found on golf course greens, tees, and fairways have turned this winter

annual weed into a year round problem. The invasion of *P. annua* can cause visible clumps of light green grass among darker grasses such as bentgrasses (*Agrostis* spp.) or Kentucky bluegrass (*Poa pratensis* L.). Damage resulting from *L. maculicollis* feeding in spring will often be in the same irregular clumping patterns along the edges of fairways where *P. annua* is densest, leaving patches of undamaged bentgrasses. Apart from undesirable aesthetic qualities, *P. annua* performs poorly in heat and drought periods during summer and requires intensive labor inputs (i.e. syringing and fungicides) to maintain healthy turf.

Management of *L. maculicollis*

Most management programs seek to control overwintering adults as they emerge from overwintering sites in adjacent rough or nearby forest leaf litter. The adults feed on the blades of grass, creating small holes or notches along the edge of the leaf blade. Further plant damage occurs when small holes are chewed into the grass plant stem where eggs are deposited. Some yellowing of the plant occurs from adult feeding. However, the majority of damage arises from the larvae emerging from the deposited eggs. It is the larval feeding and internal tunneling through the plant that will ultimately destroy the crown and kill the plant. Large larval populations of larvae can cause serious damage to stands with annual bluegrass, often leaving large visible patches of bare turf for extended periods during the season.

The first generation of larvae is usually the most destructive generation and the focus of much of the effort to control the weevil. The first-generation adults are believed to spread out further and oviposit over a greater area. The developmental stages of the

second generation are therefore even less synchronized and spread out over a larger area than in the first generation. Due to the second generation's greater spatial and temporal distribution, damage during summer is usually less severe and less localized than in spring. However, damage to *P. annua* can be extensive by the end of summer due to the combination of *L. maculicollis* feeding, disease pressure, and heat stress.

Applications of chemical insecticides, in particular pyrthrethroids, applied in the spring are currently the most efficacious means of preventatively reducing the first generation progeny and reducing densities of subsequent generations. Over the last forty years, golf course superintendents managing *L. maculicollis* have relied heavily on the use of chemical insecticides to achieve high aesthetic expectations. The increase in the number of chemical applications and the expansion of the distribution of the weevil clearly suggests that chemical management is not a sustainable approach to controlling *L. maculicollis* populations. The changes in the management practices in the last 40 years (e.g., drastic reductions in mowing height, reductions in fertilizer) have pushed turfgrass to extremes to achieve extremely high standards. It is quite possible that the same changes in turf management have contributed to the expansion of the weevil's distribution by increasing the amount of preferred hosts in the environment.

Purpose of the research

The purpose of this research was to investigate alternative control options for suppressing *L. maculicollis* populations on golf courses in the northeastern United States. The weevil continues to spread across the Northeast and Mid-Atlantic United States and eastern Canada. The overuse of insecticides and the development of resistance to broad-

spectrum materials, particularly the pyrethroid class, require that we develop alternative strategies and better understand the seasonal ecology of the pest. I have decided to focus on biological control, particularly the use of entomopathogenic nematodes to suppress *L. maculicollis* populations. Entomopathogenic nematodes have been successfully employed in managing other turfgrass pests and are easily applied by standard golf course spray equipment. Entomopathogenic nematodes are typically found in most golf course soils and in preliminary surveys have found them naturally infecting *L. maculicollis*. I focused my studies on understanding entomopathogenic nematode population dynamics within the golf course environment and the impact they have on *L. maculicollis* populations to better implement augmentative biological control. I also investigated the spatial and temporal patterns of *L. maculicollis* to develop a sampling program for golf course superintendents to better target (spatially and temporally) management efforts, whether they be biological, cultural, or chemical in nature.

CHAPTER TWO

POPULATION DYNAMICS AND INTERACTIONS BETWEEN ENDEMIC ENTOMOPATHOGENIC NEMATODES AND ANNUAL BLUEGRASS WEEVIL POPULATIONS IN GOLF COURSE TURFGRASS

Abstract

Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) are generalist obligate pathogens present in the soil of most ecosystems. They have the potential to infect a broad host range, yet the potential for endemic entomopathogenic nematodes to regulate soil-dwelling insect populations has received limited attention. I investigated the population dynamics of endemic entomopathogenic nematodes to determine their ability to regulate annual bluegrass weevil (*Listronotus maculicollis*) (Coleoptera: Curculionidae) populations, a major pest of turfgrass in the northeastern United States. Weekly sampling of nematode and *L. maculicollis* populations was conducted in untreated fairway transects on three golf courses in New Jersey between April and October of 2006 and 2007. *Steinernema carpocapsae* Weiser and *Heterorhabditis bacteriophora* Poinar were found infecting all weevil stages from third instar to teneral adults. Both nematode species exhibited a distinct seasonality, appearing in high densities in the weeks immediately following high densities of first generation weevils in the soil. A positive temporal relationship was

observed between densities of nematode-infected weevils and weevil stages between third instar and pupa, but *L. maculicollis* generational mortality due to nematode infection was highly variable between years and sites, ranging between 0 and 50%. Although infection densities and larval densities were positively correlated, *per capita* mortality did not increase with increased weevil densities. Entomopathogenic nematode distribution dynamically cycled between aggregated and uniform throughout the season across fairways. Few significant relationships were found to support the hypothesis that weevil spatial dispersion influences entomopathogenic nematode spatial dispersion. The variability in entomopathogenic nematode seasonal occurrence and generational impact on *L. maculicollis* together with the lack of spatial association with *L. maculicollis* suggest an inability of endemic entomopathogenic nematode populations to reliably regulate weevil populations on golf course fairways.

Introduction

The extent to which endemic pathogen populations impact insect populations is of great interest to applied ecologists and biological control practitioners. Pathogen populations have the ability to persist in their environments for extended periods of time in the absence of hosts and have been documented to cause crashes in insect populations (see reviews in Lacey and Kaya 2007). Despite persistence and widespread occurrence in agroecosystems little is known about pathogen field ecology, particularly their potential in regulating insect populations. Generating detailed information on the spatial and temporal dynamics of both host and pathogen are essential in the identification of key variables in pathogen-host dynamics and in evaluating a biological control agent's

potential to regulate pest densities. But these kinds of data are often not collected because of the expense and labor involved. However, a greater understanding of the abiotic and biotic factors affecting the persistence of hosts and pathogens could lead to greater predictability of epizootics and help develop guidelines for their use in conservation biological control (Lewis et al. 1998).

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) are obligate, generalist pathogens of insects, present in soils of many ecosystems around the world (Adams et al. 2006). These nematodes spend a portion of their lifecycle as a free-living, non-feeding injective juvenile (IJ), which enters its host through natural openings (mouth, spiracles, anus) or, in some cases, directly through the cuticle (Bedding and Molyneux 1982). They are classified as pathogens in biological control for, once inside the insect's hemocoel, IJs release a symbiotic bacterium that causes the host to succumb to septicemia (Kaya and Gaugler 1993). The bacteria also convert the host's tissues to suitable substrate for nematode development and reproduction. After the nematodes have developed through one to three generations and resources in the cadaver are depleted, hundreds to hundreds of thousands of IJs emerge to seek out new hosts.

Entomopathogenic nematodes have been extensively studied in the laboratory, but little is known on the dynamics of field populations (Stuart et al. 2006). Several studies have sought to determine seasonal variations in nematode population densities or describe nematode spatial dispersion (Campbell et al. 1995, 1996; Efron et al. 2001; Spiridonov et al. 2007), but little attention has been given to the nematodes' potential impact on pest populations or their use in conservation biological control. Conservation

biological control programs seek to modify or enhance the environment to increase the impact that resident natural enemies have on pest populations (Eilenberg et al. 2001). Entomopathogenic nematodes possess several attributes that make for good candidates in conservation biological control, particularly the relatively broad host range of many species which allows for persistence in the absence of target hosts and their ability to create large numerical responses following infection. The ability of a biological control agent to regulate pest populations depends on its ability to create aggregative, numerical or functional responses to increases in pest densities (Holling 1959; Hassell and May 1974). Entomopathogenic nematodes can only infect one individual and therefore are incapable of creating functional responses. However, their impact on pest populations can be significant if numerical responses can occur during the period when susceptible hosts are still present (Fenton et al. 2001). Recent theoretical models have suggested that host regulation by nematodes and long-term persistence in the system are unlikely (Fenton et al. 2000). However, entomopathogenic nematode population studies in the perennial bush lupine-ghost moth system have shown that long-term population persistence is possible (despite sensitivity to environmental conditions), and that entomopathogenic nematodes are even capable of regulating soil food webs and driving trophic cascades (Strong et al. 1996). In managed perennial systems such as turfgrass, nematode populations can exhibit temporally stable densities (Campbell et al. 1995, 1996). The implied stability of nematode populations in turfgrass suggests that they have the potential to greatly impact pest populations in this system since they overlap temporally.

The annual bluegrass weevil, *Listronotus maculicollis* Kirby is the most destructive insect pest of fine turfgrass in the northeastern United States (Vittum et al. 1999). Adult *L. maculicollis* overwinter in rough mown/tall grasses and tree line leaf litter several tens of meters away from the short mown fairways, tee boxes and greens (Diaz and Peck 2007). After emergence in late March to early April in New Jersey, adults walk to the shorter mown turfgrass areas to feed and mate (Rothwell 2003). Mated females deposit most of their eggs along the border of the rough and fairways, typically over a 3 to 4 week period (B. McGraw unpublished data). Females chew holes into the stem of the turfgrass plant to deposit their eggs. The first three larval instars feed, tunnel and develop inside the stem, largely protected from chemical controls. However, third instars are believed to occasionally move to neighboring plants. Although feeding by the young larvae may cause yellowing of the plant, it is the older larvae (fourth and fifth instars) that cause the most significant damage to the plant by tunneling through and externally feeding on the crown. This activity, when coupled with high densities, can lead to extensive damage on the edges of fairways extending over tens to hundreds of square meters. Turfgrass managers rely entirely on chemical pesticides and may apply six or more treatments per year against adult *L. maculicollis* to prevent damage from larval feeding. This pesticide overuse has led to the development of pesticide resistance in some populations (Cowles et al. 2008).

In 2005, a survey for natural enemies of *L. maculicollis* on New Jersey golf courses uncovered two entomopathogenic nematode species infecting *L. maculicollis* larvae and pupae (McGraw and Koppenhöfer 2007). *Steinernema carpocapsae* Weiser and *Heterorhabditis bacteriophora* Poinar were isolated from cadavers at several sites

and to date are the only described natural enemies of *L. maculicollis*. The main objective of this study was to describe the seasonal ecology of entomopathogenic nematodes in *L. maculicollis* infested turfgrass stands and to determine their impact on the pest population and their ability to produce aggregative and numerical responses to increasing pest densities. The long term goal is to use an improved understanding of entomopathogenic nematode field ecology for the development of nematode based biological control programs on golf courses.

Materials and Methods

Study system and design

Untreated transects were established across multiple fairways on three golf courses in central and northern New Jersey to monitor the seasonal dynamics of *L. maculicollis* and nematode populations. Transects were chosen for their history of *L. maculicollis* damage, proximity to potential *L. maculicollis* overwintering sites (areas bordering tree lines or woods) and width relative to entire fairway width (approximately 15 to 18 m across). Transects were divided into four sections beginning from the border between fairway and rough. Each section was 1.83 m long and approximately 2 m wide. In 2006 six transects were established at Pine Brook Golf Course (PB) (Manalpan, NJ), four at Brooklake Country Club (BLCC) (Florham Park, NJ) and four at Upper Montclair Country Club (UMCC) (Clifton, NJ). Two of the four transects at UMCC were inadvertently treated with insecticides at the start of the 2006 study and therefore were removed from the survey. Two new transects were added at UMCC in 2007. Soils from each transect were analyzed by the Rutgers University Soil Testing Laboratory.

Transects at PB consisted of sandy loam soils (60/27/13% sand/silt/clay composition; pH = 7.25; organic matter = 6.70 %), loam soils at BLCC (47/35/22%; pH = 5.75; OM = 13.02 %) and loamy sand at UMCC (80/17/3%; pH = 6.30; OM = 11.13 %).

Seasonal dynamics

The densities, spatial distribution and spatial association of *L. maculicollis* and entomopathogenic nematodes were monitored weekly in transects by removing four soil cores (5.4 cm diameter × 3 cm depth) from each section using a soil corer (Turf Tec International, Oakland Park, FL). To minimize contamination from nematodes in the previous sample, between samples the corer was scrubbed with a brush in dish soap water solution, rinsed with 70% ethanol, and wiped dry with paper towel. Samples were placed in a polyethylene bag, sealed, and transported to the laboratory in a cooler. All samples were taken between 0600 and 0900 h to avoid the possibility that diurnal temperature fluctuations may influence entomopathogenic nematode distribution in the soil profile (Campbell et al. 1996).

In the laboratory, samples were visually inspected for *L. maculicollis* by manually separating the soil from the plant material. The thatch and plant material was placed in a 500-ml beaker filled with 400 ml of a saturated salt solution in lukewarm water to irritate early instars, causing them to leave the plants. *Listronotus maculicollis* instars were determined based on head capsule width (Cameron and Johnson, 1971). Recovered live weevil stages were incubated individually in 24-well plates on moistened filter paper at room temperature (22–25°C) for 24 hrs, to assess viability and allow for change in color if already infected by nematodes. Infected weevils either collected from the soils, the saline solution or after the 24 hr incubation period were placed on modified

White traps (35 × 10 mm Petri dish lined with filter paper floating on tap water in a 100 × 15 mm Petri dish) (Kaya and Stock 1997) which were observed regularly to confirm cause of mortality and to harvest nematode progeny from the cadaver. Nematode species were determined after reinfection in greater waxmoth (*Galleria mellonella*) larvae, based on the color of the cadaver (orange-red for *H. bacteriophora*, beige for *S. carpocapsae*), the morphology of adult nematodes found in the dissected larvae, and the morphology of IJs emerging from the cadavers (Kaya and Stock 1997; Koppenhöfer 2007).

After examining the soil for weevil stages, the soil was pooled by replicate, thoroughly mixed, moistened (if necessary) to levels optimal for nematode activity (12–14% w/w) and placed into one deep Petri dish (100 × 25 mm) per sample. Nematodes in soil samples were exhaustively baited with *G. mellonella* larvae using standard soil aliquots (75 g). The number of IJs in the soil was correlated to the number of the infected *G. mellonella* following Koppenhöfer et al. (1998) with the exception of the use of five bait insects per round. The following equations were developed using known quantities of IJs for the endemic strains of *S. carpocapsae* and *H. bacteriophora* based on the regression of the total number of cadavers for entomopathogenic nematodes (x) versus the number of IJs penetrating (y):

$$\text{Log y } (\textit{S. carpocapsae}) = 10^{(-0.473+2.44*\log x)} \quad (2.1)$$

$$\text{Log y } (\textit{H. bacteriophora}) = 10^{(-0.420+2.11*\log x)} \quad (2.2)$$

Vertical distribution

The vertical distribution of nematodes on fairways was studied within two transects at PB. To minimize any effects of competition on vertical distribution, transects were chosen that had only one nematode species present as determined by extensive sampling prior to the start of the study. Weekly sampling began after infected *L. maculicollis* were detected in June 2006 and continued throughout the season. In 2007, sampling was conducted bi-weekly from April until larvae were detected in the soil, then weekly throughout the remainder of the season. Four cores (1.9 cm diameter × 15 cm deep) were removed from each section, aligned on a tray, and cut and pooled into sections corresponding to the top (0–5 cm), middle (5–10 cm) and bottom (10–15 cm) vertical portions. Oakfield sampler, cutting utensil, and tray were scrubbed with a brush in dish soap water solution and cleansed with 70% ethanol before sampling the next section. The samples were sealed in polyethylene bags and transported to the laboratory in a cooler. Samples were baited as previously described with the exception of using 50 g soil aliquots.

Meteorological data

Rainfall and maximum and minimum air temperature data were obtained from the National Oceanic and Atmospheric Administration (NOAA) website (www.noaa.gov) since onsite weather stations proved to be inaccurate and were often missing data for extended periods. Weather monitoring stations were selected for proximity to golf courses, with the furthest station located 9.7 km away for a particular golf course.

Data Analysis

Seasonal dynamics

Data analysis was performed using Statistix 8.0 (Tallahassee, FL 2003). Results were determined to be significant at $\alpha = 0.05$. Transformed weevil and nematode data from the seasonal survey violated assumptions of normality based on Shapiro-Wilks tests ($p < 0.001$). Kruskall-Wallis non parametric one way analysis of variance tests were therefore performed to determine if *L. maculicollis* or nematode abundance differed with respect to year, and section or area (edge sections vs. center) within transects. When significant differences were found between treatments, means were separated using the all pairwise comparisons of mean ranks procedure in Statistix. When no significant differences were found between years at the same course, the data were combined and the effect of sampling week on *L. maculicollis* captures was compared to determine peak abundance within the season using Hsu's multiple comparisons test.

Vertical distribution

The effect of depth on the abundance of entomopathogenic nematodes (within and between species) was analyzed by Kruskall-Wallis one-way non-parametric analysis of variance. Analyses were performed separately by year since the study periods differed in length. Treatment means were separated by all pairwise comparisons of the ranks of data when significant differences were detected.

Spatial dispersion and spatial association between host and pathogens

Weekly spatial dispersion values for weevils and nematodes were determined using Lloyd's (1967) mean crowding (LMC) since it generates a dispersion value that is biologically relevant and has been used in past entomopathogenic nematode population

studies (Campbell et al. 1996; Spiridonov et al. 2007). Dividing LMC by the sample mean results in an index of aggregation referred to as patchiness (X*/x). Patchiness is the number of times more crowded an individual in a sample is than an individual in a randomly dispersed population with the same mean. Patchiness values of less than zero, zero and greater than zero indicate uniform, random and aggregated distributions, respectively. Mean crowding values were linearly regressed against density to ensure that the index was unbiased. Covariance coefficients for density and patchiness were determined for each nematode species by course and year as well as in each of the 26 individual data sets (12 and 14 transects in 2006 and 2007, respectively).

I sought to determine if significant relationships exist between the covariance of either spatial dispersion (LMC value) or density of either entomopathogenic nematode and *L. maculicollis*. *Listronotus maculicollis* density and weekly dispersion values were analyzed for covariance with nematode values for five time-lagged data sets (t = 0, 1, 2, 3, 4 wk) using non-parametric correlation tests. The range of the lag covers approximately two cycles of infection to emergence (Brown and Gaugler 1997) and was chosen to allow for the effect of *L. maculicollis* dispersion to be detected with nematode recycling. *Listronotus maculicollis* counts were combined for all soil stages (fourth instar through pupa). Spearman rank correlation was employed since the data violated the assumption of normality and for the possibility that the relationship might not be linear. Analyses were performed on datasets with a minimum of four paired observations.

Generational Mortality of *L. maculicollis*

The impact of nematodes on *L. maculicollis* was measured by partial life table analysis. Generational mortality was calculated based on the observed number of infected third instars through pupae and calculated as:

100% - [100% × (proportion surviving infection in third instar) × (proportion surviving infection in fourth instar) × (proportion surviving infection in fifth instar) × (proportion surviving infection in pupa)] (2.3)

Results

Seasonal Dynamics

Listronotus maculicollis

The phenology of *L. maculicollis* is depicted in Fig. 2.1. The magnitude of population fluctuations differed between years, but there was no significant effect of year on the abundance of adults or all *L. maculicollis* immature stages combined in any population (*P* values ranging from 0.07 to 0.96), and therefore data from 2006 and 2007 were combined. Courses differed in the relative abundance of first generation larvae, even though densities of the emerging overwintered adults were relatively consistent among sites. At all courses a significantly higher abundance of first through third instars (within-plant stages) was observed in mid-May to early June (first generation) than at the two following peaks (second and third generation). Weevil stages present in the soil peaked between 25 May and 19 June, with the southernmost population (PB) peaking earliest. BLCC supported the largest densities of weevils in both years. Older larvae

were present in the soil, and therefore most likely to encounter entomopathogenic nematodes, from 23 May to 26 September in 2006 and between 29 May to 9 October in 2007. UMCC, though lower in relative density, supported soil stages of weevils from 28 May to 26 September in 2007.

Patchiness values indicated that all *L. maculicollis* stages were significantly more aggregated than a random distribution. No significant differences were observed between larval stages by habitat although soil stages trended towards a greater aggregation (F = 2.66; df= 1,179; P = 0.10). An effect of section on *L. maculicollis* seasonal abundance was only observed at PB. Significant F values were obtained for several stages; however, means separations were not always possible. All stages trended towards greater numbers on the edges of fairways than in the centers; however, only when the means of all immature weevil stages were combined did edge sections have greater densities than center sections.

Entomopathogenic nematodes

Steinernema carpocapsae and *H. bacteriophora* were the only nematode species detected in the surveys of fairways between the 2005 study (B. McGraw unpublished data) and 2006 and 2007 study presented here. Nematode density fluctuated throughout each season (Fig. 2.2) and between seasons, though exhibiting strong seasonal peaks following the period when *L. maculicollis* soil stages were most abundant (mid to late June). *Steinernema carpocapsae* and *H. bacteriophora* were also found in high densities in late August and September when weevil densities were low, suggesting that other insect species serve as nematode hosts on fairways. *Steinernema carpocapsae* had several

abundance peaks during the season, often when weevils were absent, indicating the importance of other arthropods in its population dynamics on fairways.

The instability of the golf course turfgrass system is reflected in the proportion of nematode-positive samples (Fig. 2.3). In 2006, a very high percentage (50–100%) of the samples taken in June/early July contained nematodes. The proportion dramatically declined when soil moisture decreased and temperature increased above 30 °C during drought like conditions of July and August. Populations rebounded slightly in September, as rainfall increased. In 2007, nematode densities at all sites were extremely low between mid-April and early June after an extreme rainfall event (250 mm in 48 h) but thereafter sharply increased to peak densities.

Strong significant differences ($P < 0.01$) in the abundance of nematodes were found between golf courses and years. *Steinernema carpocapsae* declined and *H. bacteriophora* increased at PB in 2007. Both nematode populations at BLCC increased from 2006 to 2007. *Steinernema carpocapsae* tended to be recovered in greater densities than *H. bacteriophora*, yet due to the high variability in nematode counts, the difference was not always statistically significant. At UMCC *H. bacteriophora* was typically more abundant than *S. carpocapsae. Steinernema carpocapsae* and *H. bacteriophora* overlapped in time and space, both present within the same sample 59 times out of the 671 nematode-positive samples between 2006 and 2007. The majority of mixed samples were observed at PB (69%) where nematode populations, especially *S. carpocapsae*, were found in high densities.

Significant differences in nematode abundance were found by sections, but without clear trends unifying the data between sites. *Steinernema carpocapsae* was most

often recovered from the centers of fairways at PB ($H = 18.9$; $df = 1, 1015$; $P < 0.0001$), with the opposite being true at BLCC ($H = 25.1$; $df = 1, 847$; $P < 0.0001$). *Heterorhabditis bacteriophora* seemed to be less affected by location, with only BLCC's population being more abundant in centers of fairways than in edges ($H = 4.4$; $df = 1, 847$; $P = 0.03$).

Vertical distribution

Nematodes were most abundant at the start of the vertical distribution survey in 2006, following initial weevil infections. *Heterorhabditis bacteriophora* was present in the lower strata in low densities during the excessively dry periods of July and August, but *S. carpocapsae* went entirely undetected through this period. *Steinernema carpocapsae* reappeared in late August, possibly re-colonizing from off-fairway areas or through delayed emergence from hosts (Brown and Gaugler 1997; Koppenhöfer et al. 1997). In 2007 nematodes were detected in early April, then disappeared in all vertical strata after the heavy rainfall, rapidly increased in mid June, and finally declined sharply in summer.

Steinernema carpocapsae and *H. bacteriophora* were recovered in all vertical strata, though the highest densities of both species were found in the top stratum where *L. maculicollis* are present (Fig. 2.4). In both 2006 and 2007 significantly more *S. carpocapsae* were found at 0–5 cm ($F \geq 8.85$; $df = 2, 346$; $P > 0.002$) than at 10–15 cm. No significant differences where found in the abundance of *S. carpocapsae* between 0–5 cm and 5–10 cm despite seasonal average densities at the surface being 300- and 50-fold greater in 2006 and 2007, respectively. *Heterorhabditis bacteriophora* densities were not significantly affected by soil depth in either year of the study ($F \geq 0.49$; $df = 2, 346$; $P >$

0.49), even though 8- to 10-fold higher average densities were observed at 0–5 cm than at 5–10 cm and 10–15 cm depth. Significantly more *H. bacteriophora* were detected at 10–15 cm when compared to *S. carpocapsae* in both years of the study ($F \geq 4.85$; $df = 1, 68$; $P > 0.02$). Additionally, in 2007 higher densities of *H. bacteriophora* were observed at 5–10 cm. *Steinernema carpocapsae* was observed in significantly greater densities than *H. bacteriophora* at 0–5 cm in 2006 ($F \geq 5.80$; $df = 1, 48$; $P > 0.02$) but not in 2007.

Effect of entomopathogenic nematodes on *L. maculicollis* population dynamics

Heterorhabditis bacteriophora and *S. carpocapsae* infected all stages of *L. maculicollis* between third instar and teneral adult. I frequently observed each species infecting different individuals of the same stage within the same transect or section throughout the season which may suggest that the two nematode species compete for resources. The generational mortality caused by nematode infections ranged from 0 to 50% (Tables 2.1 and 2.2), with the highest number of infections observed during the first generation in both years. The *per capita* impact on weevil populations was the greatest against the first generation in 2006 but against the second generation in 2007. *Heterorhabditis bacteriophora* was the dominant species infecting *L. maculicollis* in the preliminary survey of New Jersey golf courses in 2005 (98% of infected cadavers), but more intensive sampling in 2006 revealed a more balanced contribution from each species (55% *H. bacteriophora*). In 2007, low nematode densities in May led to few infections against the first *L. maculicollis* generation. Though densities of both nematode species were extremely low, a relatively lower number of first generation weevil infections by *S. carpocapsae* compared to *H. bacteriophora* resulted in a greater percentage of *H. bacteriophora* infections across the season (76%).

A temporal relationship was observed between the number of infected *L. maculicollis* and the combined densities of *L. maculicollis* stages from third instar to pupa (Fig. 2.5). The number of infections increased with increasing weevil densities, yet no relationship was observed between the densities of *L. maculicollis* larvae and the proportion of weevils infected (Fig. 2.6).

Spatial dispersion and spatial association between host and pathogens

Nematode patchiness values indicate that nematode spatial dispersion is far more dynamic than what has been previously reported. In transects with consistently high enough nematode numbers to obtain weekly dispersion values, *H. bacteriophora* and *S. carpocapsae* spatial distribution cycled between aggregated and uniform in a matter of weeks (Fig. 2.7). No relationships were detected from the linear regression of LMC values (X*) and density of *S. carpocapsae* and *H. bacteriophora* in samples ($r^2 = 0.01$ and 0.05, respectively) suggesting that patchiness was an unbiased index of dispersion. Despite this, negative patchiness values (uniformity) were observed only when nematode densities averaged less than one nematode per 50 g of soil, which often occurred in the week following a date when no nematodes were observed in a transect. However, removing the uniform spatial values from the linear regression of LMC versus density only strengthened the support for patchiness being an unbiased index for *S. carpocapsae* and *H. bacteriophora* dispersion ($r^2 = 0.01$ and 0.0002, respectively).

Few significant relationships were found to support the hypothesis that nematodes are spatially associated with *L. maculicollis*. Out of the 26 datasets, only 12 for *H. bacteriophora* and 14 for *S. carpocapsae* had enough paired observations to conduct covariance analyses. Statistically significant covariance was found in no more than three

datasets for a given time lag, occasionally with mixed relationships. *Heterorhabditis bacteriophora* patchiness with a 2 wk lag was negatively associated in one dataset and positively in two datasets, with one strongly significant positive result ($P < 0.01$). Overall, *H. bacteriophora* datasets produced more significant covariance results than *S. carpocapsae* for both patchiness (5 vs. 1) and density (4 vs. 2). *Heterorhabditis bacteriophora* dispersion was negatively correlated with *L. maculicollis* stages initially (t = 0, 1 wk) and positively with greater delays (t = 2, 4 wk). Density was generally positively correlated with *L. maculicollis* density with the exception of the 4 wk time lag.

Discussion

This study demonstrates that endemic populations of *S. carpocapsae* and *H. bacteriophora* infect a majority of *L. maculicollis* stages and can cause moderate reductions in the host's populations. The endemic populations possess many qualities that make them amenable for use in conservation biological control, including increasing levels of infection with increasing *L. maculicollis* densities, recycling in hosts, and persistence in the environment. The moderate generational mortality caused by nematodes observed in this study may have been due to the fact that broad spectrum chemical pesticides were withheld only in a relatively small area, with no other habitat manipulation. Since this study sought to address the potential impact of native entomopathogenic nematode populations on *L. maculicollis* densities one can only speculate about their use in the conservation biological control of the weevil and identify areas where further habitat modification (e.g., increased mowing height, soil modifications, encouraging non-pestiferous soil insect hosts, modification over a larger

scale or longer period of time) might stabilize their densities and lead to greater levels of *L. maculicollis* reduction. However, nematode sensitivity to temperature and moisture extremes in turfgrass appears to limit nematode persistence at high densities. Dramatic seasonal fluctuations and a lack of spatial association with hosts decrease the predictability of the nematodes' impact on *L. maculicollis* and warrant further investigation into how they may be manipulated to improve consistency.

For biological control to be successful, agents must suppress host populations to low and stable densities (Murdoch et al. 1985). The ability of generalist predators to impart this level of regulation in a classical or conservation context has been widely debated (see reviews in Symondson et al. 2002). However, populations of generalist predators, such as nematodes, might be better suited for conservation biological control for their ability to persist or recycle through other insects when pests are absent. Also, research has shown that when pests are the dominant hosts, generalists can respond strongly to increases in densities (Bohan et al. 2000; Winder et al. 2005). A temporal correlation between infected *L. maculicollis* and increasing *L. maculicollis* densities was observed. However, the proportion of infected weevils did not increase when the combined densities of *L. maculicollis* stages between third instar and pupa increased above 50 per 0.09 m^2. The saturation effect of proportional mortality and the inability of nematodes to create a functional response demonstrate the limitations of the current populations to adjust to increasing prey densities. Future research is needed to determine how to manipulate insect populations on fairways, allowing for high numbers of nematodes to build up prior to the invasion of *L. maculicollis*.

Since nematodes are limited in their mobility, their potential for regulating *L. maculicollis* diminishes if they do not overlap spatially. Little evidence was found for spatial association, measured in densities or spatial values, between *L. maculicollis* and either nematode species. Nematodes have the potential to generate large numerical responses following infections, yet incorporating a time lag to allow for recycling in hosts did not improve the predictability of spatial dependence. Similarly, Efron et al. (2001) and Campbell et al. (1995) did not detect spatial dependence in their observational studies. Lack of spatial dependence could suggest that the sampling regime was ineffective at detecting the dependence or that encounters of natural populations of nematodes and insect hosts are rare and unlikely to regulate *L. maculicollis* populations over the scale studied.

Nematode dispersion across the seasons was highly aggregated as previously reported in numerous systems (Campbell et al. 1995, 1996, 1998; Efron et al. 2001; Spiridonov et al. 2007). Nematode aggregation can be caused by patchy host distribution coupled with high reproductive capabilities and limited mobility. Unique to the findings presented here was that weekly nematode dispersion values at the scale of several square meters fluctuated from aggregated to random in very short time frames (1–2 wk). The cycling of dispersion demonstrate that entomopathogenic nematodes probably exist as complex metapopulations within an area of several square meters, fluctuating from uniform or random to aggregated in response to migration, immigration, death, infection and reproduction (Stuart et al. 2006; Spiridonov et al. 2007).

A dramatic yet consistent annual fluctuations in population density was observed in this study, unlike previous studies in sod turfgrass within the same region. Campbell

et al. (1995) observed stable entomopathogenic nematode densities when averaged across the season, leading some authors and reviewers to conclude that entomopathogenic nematodes in temperate areas lack seasonal fluctuations (Hominick and Briscoe 1990; Lewis et al. 1998; Boag and Yeates 2004; Barbercheck and Hoy 2005). Differences between the population dynamics of entomopathogenic nematodes in golf course fairways and sod could be due to several abiotic and biotic factors. Fairway turfgrass is maintained at lower heights than sod which could affect its capacity to support seasonally consistent nematode densities and hosts. Soil temperature and moisture tend to fluctuate more under lower mown turf stands than in higher mown areas. Temperature and moisture extremes can be detrimental to nematode movement (Molyneux and Bedding 1984; Gaugler 1988) and infectivity (Molyneux and Bedding 1984; Grant and Villani 2003; Koppenhöfer and Fuzy 2007). Conversely, low soil moisture levels can improve nematode persistence (Kung et al. 1991; Grant and Villani 2003; Koppenhöfer and Fuzy 2007).

Effects of low soil moisture on entomopathogenic nematode dynamics have been observed in other field surveys. Efron et al. (2001) reported higher abundance of entomopathogenic nematodes in Israeli citrus groves during fall and winter when hosts were present and soil moisture and temperature were conducive to nematode survival. The effect of wet and dry cycles has also been shown to be a key factor in the entomopathogenic nematode induced tri-trophic cascades in natural systems (Preisser and Strong 2004). In this study, high temperatures appeared to greatly affect the abundance of both nematode species, especially of the surface active *S. carpocapsae*. *Steinernema carpocapsae* was undetected on fairways, both in vertical and horizontal sampling at PB

in July and August 2006, but re-colonized transects in early September when conditions improved. I observed low densities in all vertical strata during times when surface temperatures were above thermal optimums for nematode reproduction and survival (Grewal et al. 1994a), which was consistent with the findings of García del Pino and Palomo (1997). *Heterorhabditis bacteriophora* was found in greater densities in the lower soil strata than the surface active *S. carpocapsae* between July and August when temperatures were observed to exceed 30°C. Conversely, heavy rainfall may have had a dramatic effect on the abundance and persistence of both nematode species. Extreme rainfall in a 48 h period in mid-April appeared to strongly suppress densities until the seasonal peaks in mid June. Nematode movement in water-saturated soils is generally restricted or inhibited (Wallace 1971; Koppenhöfer and Fuzy 2007) but it remains unclear as to why populations remained depressed for such extensive lengths of time.

Steinernema carpocapsae and *H. bacteriophora* were the only entomopathogenic nematode species detected in soil samples and infecting *L. maculicollis* in the 3 years of surveys of golf course fairways. Both species were often detected within the same transect, and occasionally within the same sample, suggesting that they compete for the same resources throughout the season. Multiple entomopathogenic nematode species have been shown to coexist in nature (Akhurst et al. 1992; Stuart and Gaugler 1994; Campbell et al. 1995; Millar and Barbercheck 2001; Lawrence et al. 2006; Spiridonov et al. 2007). Competition theory states that two species that co-exist while sharing the same resource should evolve niche partitioning to avoid extinction (Armstrong and McGehee 1980). Several hypotheses have been posed as to what mechanisms allow entomopathogenic nematodes to coexist including seasonal variation in environmental

conditions, temporal variation in abundance, resource abundance and foraging strategy (Kaya and Koppenhöfer 1996; Koppenhöfer and Kaya 1996). I did not detect partitioning by either species in the life stages of *L. maculicollis* that were infected or in infections in time. Although both species were highly seasonal their peak densities occurred during the same period of time further increasing the likelihood of an interaction. This leads me to believe that either hosts were not limiting or behavioral attributes such as foraging strategies sufficed to allow segregation and co-existence. The foraging strategies of the two observed nematodes species are quite different. *Steinernema carpocapsae* employs an "ambusher" strategy, attacking surface active insects, whereas *H. bacteriophora* employs "cruiser" strategy, attacking sedentary prey throughout the soil profile (Campbell and Gaugler 1997; Lewis et al. 2006).

This study provides insight into the population dynamics of entomopathogenic nematodes in golf course turfgrass and highlights several factors that hinder their reliability as conservation biological control agents. Although relying on natural populations to reduce pest populations could be risky due to the inherent variability in epizootic outbreaks (Lacey et al. 2001), understanding the population dynamics of nematodes could improve the predictability of epizootics to occur or at the very least determine how nematodes could be better utilized in inundative releases. We are increasing our understanding of the types of direct and indirect effect that management practices have on nematodes in golf course turfgrass (Koppenhöfer and Grewal 2005; Alumai et al. 2006), but future work will need to address effects on non-target insects that might serve as alternate hosts for nematodes. Conserving the insect community early in the season by reducing broad spectrum insecticide applications in space and time could

lead to more consistent and possibly greater densities of nematodes that may more reliably reduce *L. maculicollis* populations.

Table 2.1. Summary of *L. maculicollis* generational mortality attributable to nematode infection at Brooklake Country Club (BLCC), Pine Brook Golf Course (PB) and Upper Montclair Country Club (UMCC) in 2006.

Generation	Stage	BLCC		PB		UMCC	
		Total (n)	Infected (%)	Total (n)	Infected (%)	Total (n)	Infected (%)
1st	3rd instar	68	0.0	24	8.3	13	7.7
	4th instar	145	7.6	54	20.4	44	11.4
	5th instar	166	10.8	21	19.0	35	11.4
	Pupa	118	2.5	41	4.9	28	3.6
Generation mortality[a]			19.7		43.8		30.1
2nd	3rd instar	22	4.5	4	0.0	4	0.0
	4th instar	48	8.3	2	0.0	2	0.0
	5th instar	42	16.7	1	0.0	1	0.0
	Pupa	41	0.0	2	0.0	2	0.0
Generation mortality			27.1		0.0		0.0
3rd	3rd instar	10	0.0	0	n/a[b]	0	n/a
	4th instar	16	6.3	0	n/a	0	n/a
	5th instar	33	3.0	0	n/a	0	n/a
	Pupa	24	0.0	0	n/a	0	n/a
Generation mortality			9.1		n/a		n/a

[a]Generational mortality = 100% - [100% × (proportion surviving infection in third instar) × (proportion surviving infection in fourth instar) × (proportion surviving infection in fifth instar) × (proportion surviving infection in pupa)].
[b] n/a = not applicable

Table 2.2. Summary of *L. maculicollis* generational mortality attributable to nematode infection at Brooklake Country Club (BLCC), Pine Brook Golf Course (PB) and Upper Montclair Country Club (UMCC) in 2007.

Generation	Stage	BLCC		PB		UMCC	
		Total (n)	Infected (%)	Total (n)	Infected (%)	Total (n)	Infected (%)
1st	3rd instar	14	0.0	27	0.0	7	0.0
	4th instar	38	5.3	29	3.4	13	7.7
	5th instar	124	3.2	58	1.7	56	0.0
	Pupa	82	4.9	67	1.5	63	1.6
Generation mortality[a]			12.7		6.4		9.8
2nd	3rd instar	28	7.1	3	33.4	12	8.3
	4th instar	37	10.8	3	0.0	20	5.0
	5th instar	87	18.4	7	14.3	60	13.3
	Pupa	69	8.7	3	0.0	35	14.3
Generation mortality			38.3		42.4		35.1
3rd	3rd instar	35	2.9	3	0.0	25	0
	4th instar	35	17.1	2	0.0	8	12.5
	5th instar	75	20.0	3	0.0	25	8.0
	Pupa	60	23.3	6	0.0	12	8.3
Generation mortality			50.4		0.0		26.2

[a] Generational mortality = 100% - [100% × (proportion surviving infection in third instar) × (proportion surviving infection in fourth instar) × (proportion surviving infection in fifth instar) × (proportion surviving infection in pupa)].

Figure 2.1. Seasonal phenology of *Listronotus maculicollis* at Brooklake Country Club (BLCC), Pine Brook Golf Course (PB) and Upper Montclair Country Club (UMCC) (2006–2007).

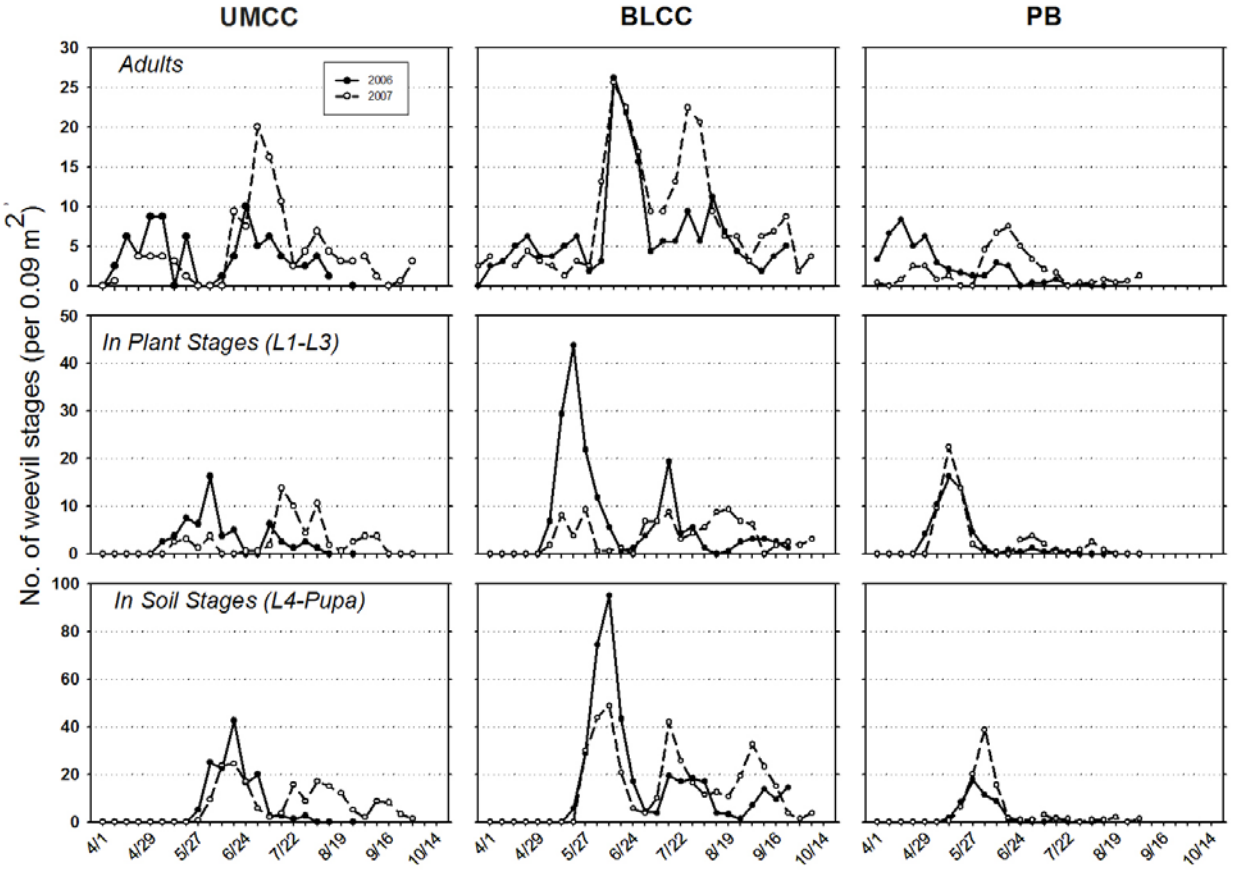


Figure 2.2. Seasonal phenology of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* at Brooklake Country Club (BLCC), Pine Brook Golf Course (PB) and Upper Montclair Country Club (UMCC) (2006 to 2007).

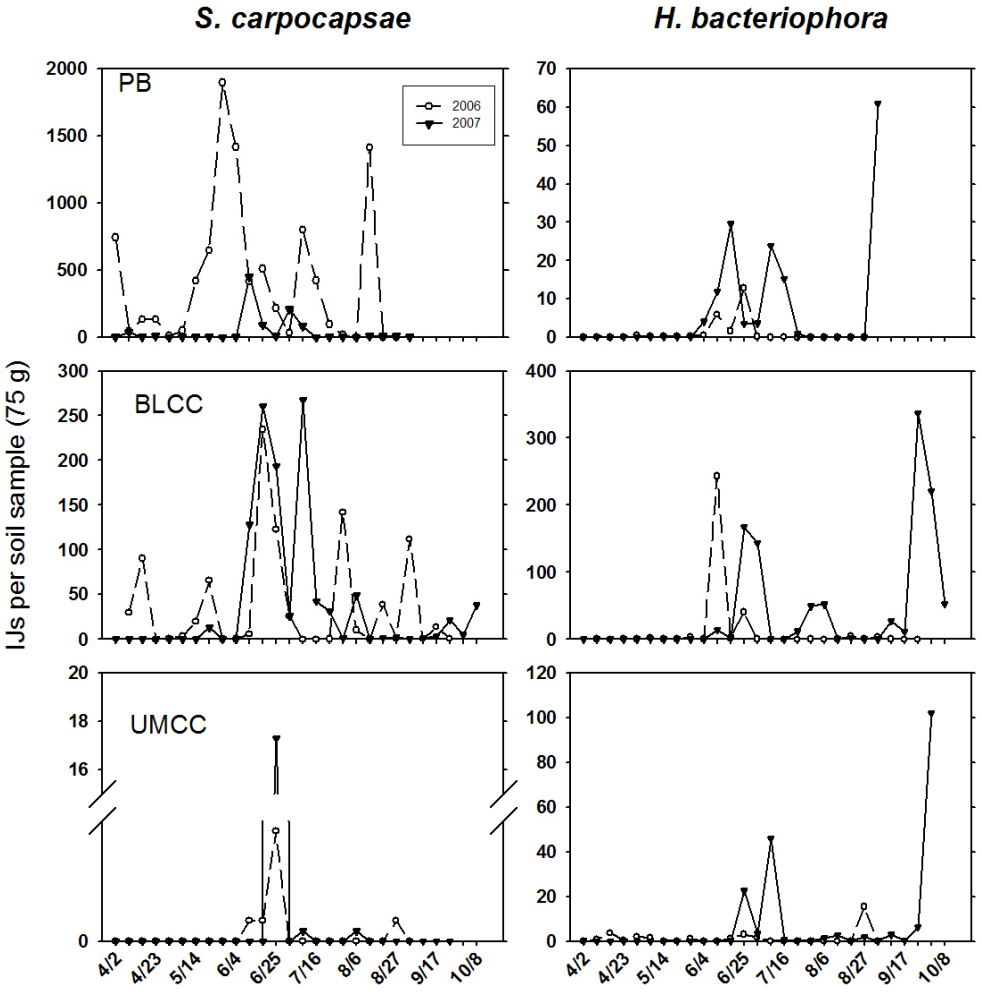


Figure 2.3. Percentage of soil samples containing nematodes and meteorological data (2006 to 2007).

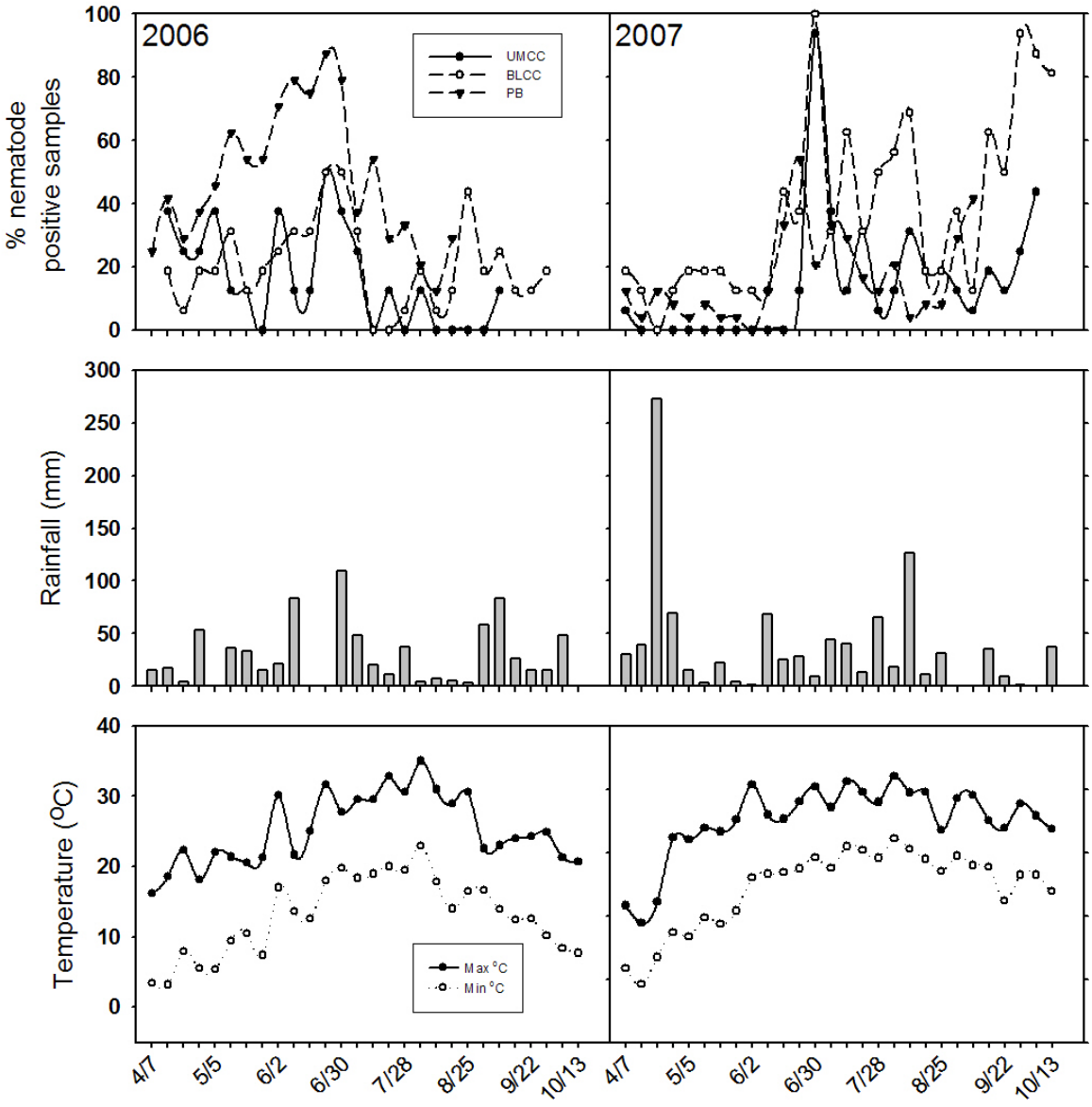


Figure 2.4. Vertical distribution of *Steinernema carpocapsae* and *Heterorhabditis* on two separate fairways at Pine Brook Golf Course (PB) (2006 to 2007). Error bars represent the standard error of the mean.

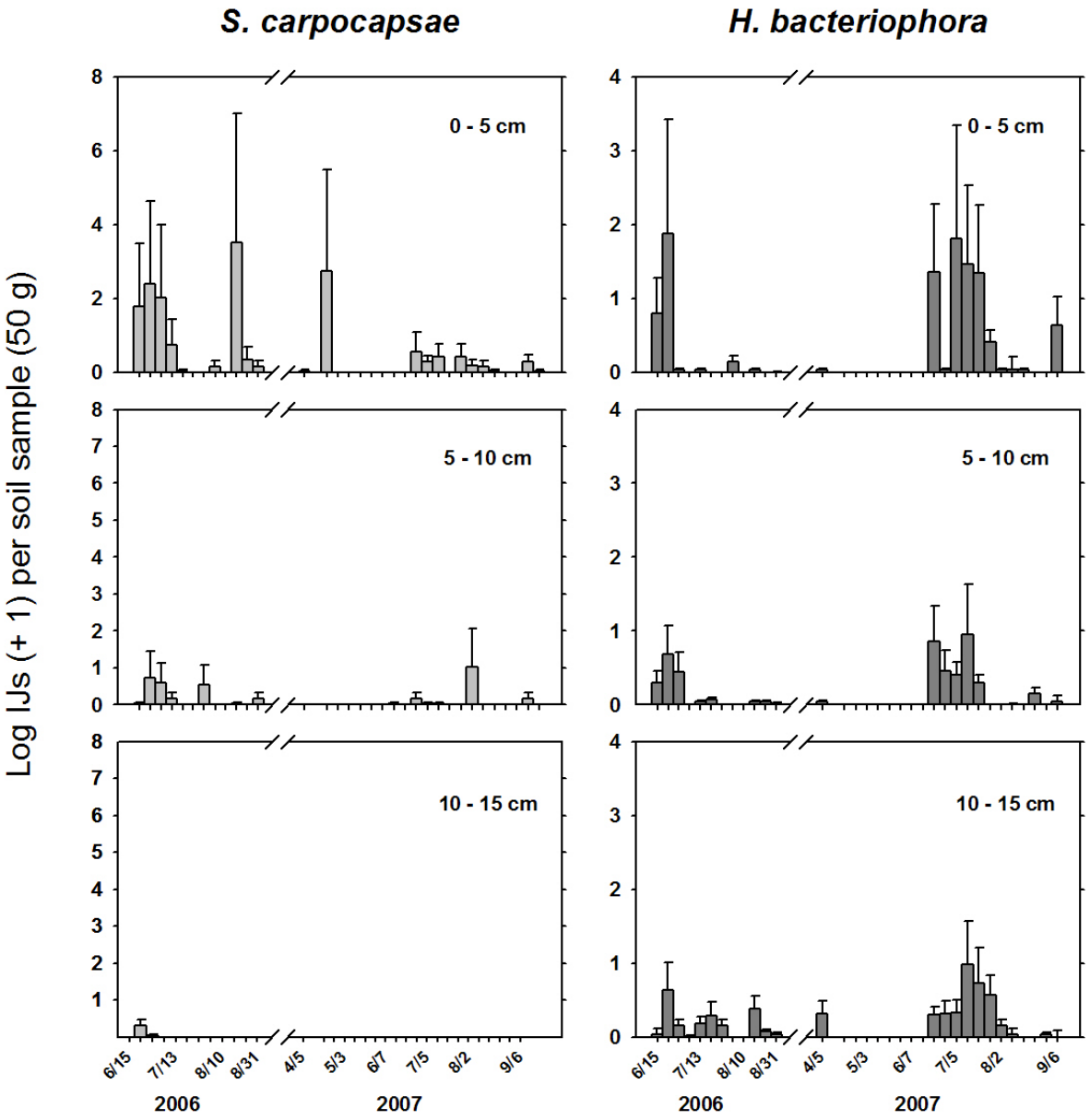


Figure 2.5. Temporal relationship between numbers of nematode-infected individuals and *Listronotus maculicollis* third instar through pupa densities (2006–2007).

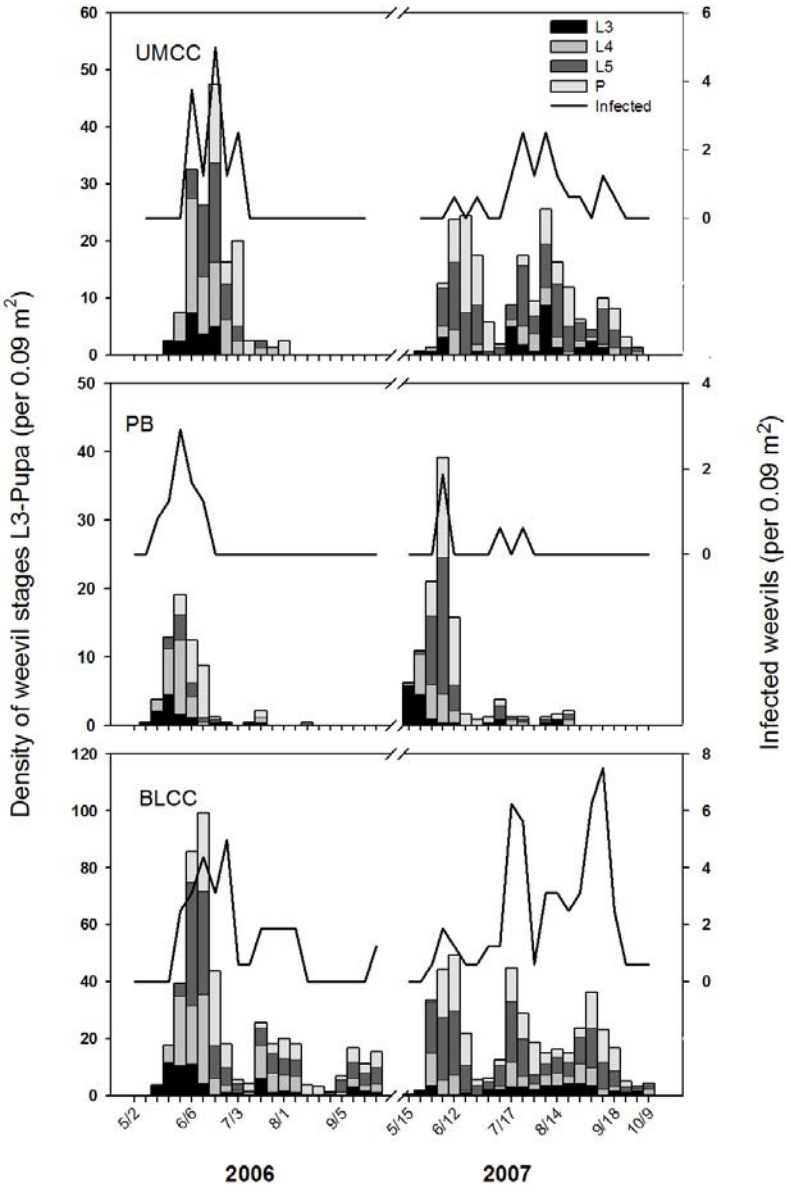


Figure 2.6. Relationship between *Listronotus maculicollis* densities (third instar through pupae combined) and the number and proportion of infected weevils.

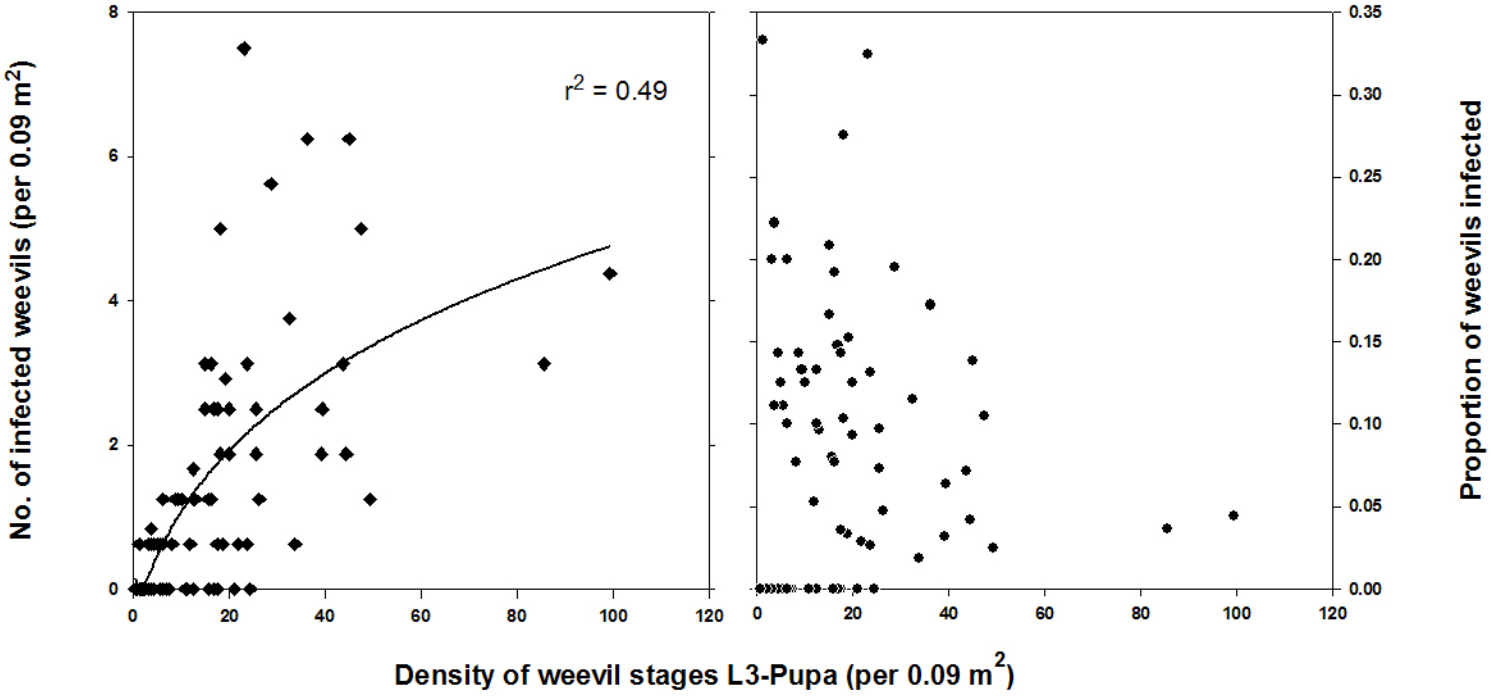
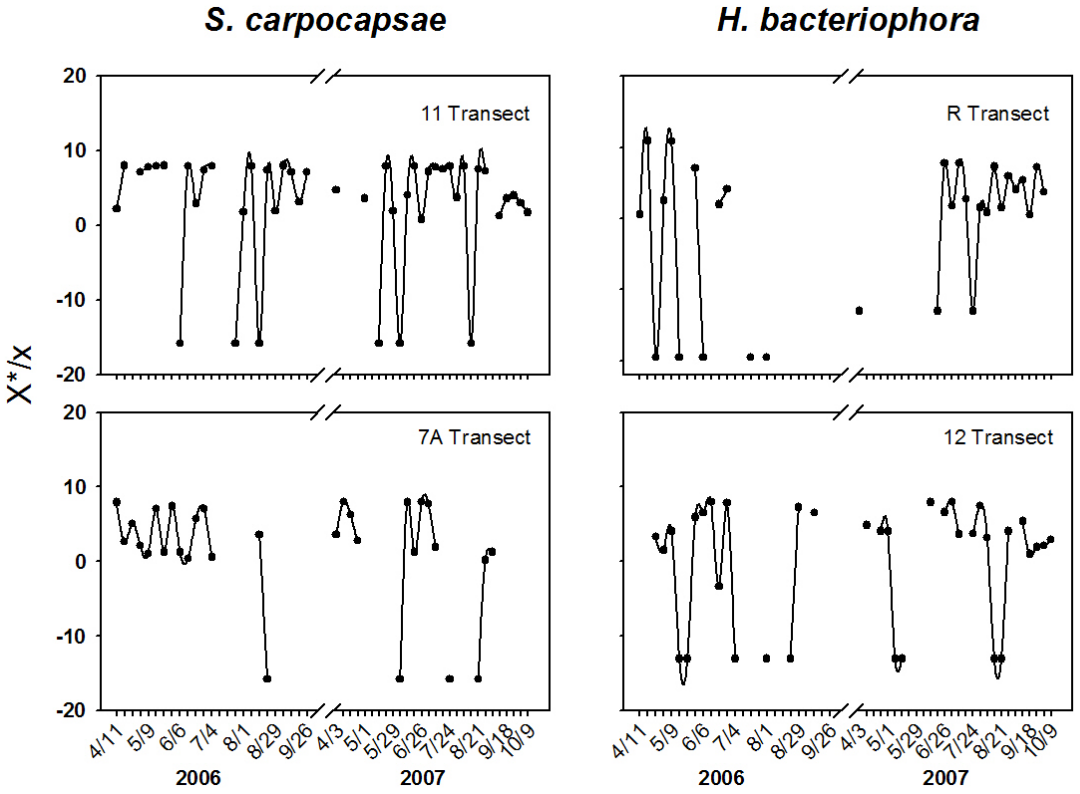


Figure 2.7. Temporal patchiness (X*/x) of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* in four transects (2006–2007). Values greater than, equal to, and less than 0 indicate aggregated, random or uniform nematode populations, respectively.



CHAPTER THREE

EVALUATION OF TWO ENDEMIC AND FIVE COMMERCIAL ENTOMOPATHOGENIC NEMATODE SPECIES (RHABDITIDA: HETERORHABDITIDAE AND STEINERNEMATIDAE) AGAINST ANNUAL BLUEGRASS WEEVIL (COLEOPTERA: CURCULIONIDAE) LARVAE AND ADULTS

Abstract

The annual bluegrass weevil, *Listronotus maculicollis,* is a highly destructive pest of golf course turfgrass in the northeastern United States. The virulence of entomopathogenic nematodes to larvae and adults of *L. maculicollis* was assessed as an initial step in developing a biological control program for this pest. Two endemic and five commercially available nematode species were tested in soil and field-infested turf core assays. Adult susceptibility was generally low to moderate (11-65% mortality) and was not affected by the age or overwintering condition of the insect. A mixture of two nematode species with different foraging behaviors (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) achieved the highest levels of adult control, but required 6 d to obtain 50% mortality at five times standard field application rate (125 nematodes/cm^2). Conversely, fourth- and fifth- instar larvae were highly susceptible to nematodes. *S. feltiae, S. carpocapsae* and *S. kraussei* significantly reduced fourth instars in both years of the study. The same three species along with the commercial strain of *H. bacteriophora* significantly reduced high densities of fifth instars to below field damage

thresholds. In general, *Steinernema* spp. trended towards greater control than *Heterorhabditis* spp. for both fourth and fifth instars. No difference was observed among the virulence of endemic and commercial nematode strains to any *L. maculicollis* stage tested. The data indicate that *Steinernema* spp. could provide high levels of control when applied curatively against *L. maculicollis* larvae.

Introduction

The annual bluegrass weevil, *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is the single most destructive pest of fine turfgrass in the northeastern United States (Vittum et al. 1999). Damage typically occurs to short mown stands of golf course, tennis and lawn bowling turfgrass with high percentages of annual bluegrass, *Poa annua* L. Adult *L. maculicollis* migrate from overwintering, forest litter sites on to short mown areas during April in New Jersey, where they feed and mate over the course of several weeks. The female deposits her eggs directly into the stem of the turfgrass plant in which the emerging larvae develop through the third instar (Cameron and Johnson 1971). Older larvae and pupae are found in the soil-thatch matrix where the larvae feed externally on the crown, ultimately destroying the actively growing point of the turfgrass plant. Damage from larval feeding can be extensive if large densities of larvae are left unchecked.

Currently, the most efficacious means of controlling the weevil is through broad-spectrum chemical pesticides applied preventively against the overwintered adults before the females start laying eggs in spring. Spring densities of adults in the epicenter of the weevil's distribution (metropolitan New York City area) can be very high and the fear of

turf loss so great, that turfgrass managers often make multiple applications to each of the three generations, usually without assessing the threat of damage. Overuse and poor timing of pyrethroid applications have led to the development of resistant weevil populations (Cowles et al. 2008). The development of alternative, ecologically sensitive controls and the mitigation of resistance issues have become a priority for turfgrass researchers in the northeastern United States.

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) are obligate parasites of a diverse array of insects and are present in the soils of many ecosystems worldwide. These nematodes possess a unique life cycle, a portion of which is spent outside of the host as a non-feeding infective juvenile (IJ). IJs penetrate hosts through natural openings (mouth, spiracles and anus) or, in some instances, directly through the cuticle. Once inside the insect's hemocoel, the IJs release a symbiotic bacterium that they harbor within their intestine, which cooperates with the nematode to overcome the host defenses and ultimately kill the host but also retards growth of microbial competitors. Entomopathogenic nematodes are attractive biological control agents for their ability to cause rapid death, generate large numerical responses through reproduction, and persist and cause future reduction in pest densities. Nematodes can also be integrated into traditional pest management programs since they can be applied in standard spray equipment, are compatible with many agrochemicals (Koppenhöfer and Grewal 2005), and, in some instances, interact synergistically with chemical pesticides (Koppenhöfer et al. 2000).

Listronotus maculicollis spend most of their life cycle and a significant portion of the year in the soil or on the soil surface, and therefore have been identified as a potential

target for biological control using entomopathogenic nematodes (Georgis et al. 2006). Three separate field studies of *L. maculicollis*-infested golf course fairways have detected nematodes infecting *L. maculicollis*, which to date are their only described natural enemies. Vittum (1980) described a *Neoplectana* (= *Steinernema*) species infecting *L. maculicollis* larvae and pupae, Grant and Rossi (2004) found *Heterorhabditis bacteriophora* Poinar infected weevils in studies at a reduced chemical management golf course, and McGraw and Koppenhöfer (2007) detected epizootics of *H. bacteriophora* in weevil populations at two courses in New Jersey. More intensive sampling of *L. maculicollis* populations in New Jersey suggested that both *H. bacteriophora* and *Steinernema carpocapsae* Weiser frequently contribute to reducing *L. maculicollis* densities (McGraw and Koppenhöfer 2008a).

Previous research using entomopathogenic nematodes to suppress *L. maculicollis* was exclusively conducted in field trials, bypassing screening of candidates under controlled conditions. Commercially available *S. carpocapsae* has demonstrated low to high control (22–98%) when applied curatively against larvae and low levels against adults (Vittum 1995, see review in McGraw and Koppenhöfer 2007). The limited and variable data have warranted a closer examination of the potential of nematodes as biological control agents, particularly identifying better adapted species, which could improve upon the variability or the level or control, if not both. In the present study, I evaluated susceptibility of *L. maculicollis* stages to the nematodes under laboratory conditions, with the overall goal to determine the feasibility of suppressing populations on golf courses. The objectives of the study were threefold: 1) determine which *L. maculicollis* stages are most susceptible to nematodes, 2) identify the most virulent

commercial nematode species for future field releases, and 3) determine if field-isolated, endemic nematode strains have greater virulence than the conspecific commercial strains. The information gained from this study should identify the nematode species most likely to control *L. maculicollis* in large-scale releases on golf courses.

Materials and Methods

Nematodes

Nematode species, strains/isolates, sources, abbreviations and assays in which they were used are listed in Table 3.1. Nematodes were reared in parallel in late instar greater wax moth, *Galleria mellonella* L., larvae at room temperature (22–25°C), with the exception of *Steinernema kraussei* Filipjev which was reared in an incubator at 20°C. *G. mellonella* were supplied by Northern Bait (Chetek, WI), Nature's Way (Harrison, OH) and Morning Dew Bait (Trumansburg, NY). IJs were harvested as they emerged from nematode-killed insects and stored at 10°C. After 2 wk of emergence, IJs were pooled and used in bioassays the following week.

Insects

Adult *L. maculicollis* were collected from either overwintering sites prior to winter or on fairways after they had emerged from overwintering sites in spring. Overwintering adults were collected on 12 November 2005 from beneath forest leaf litter adjacent to untreated, infested fairways at Pine Brook Golf Course (Manalapan, NJ). The weevils were extracted in the field by taking the top 2 to 3 cm of soil, placing the soil in 19-liter buckets and adding lukewarm water (25-30^{o}C). A piece of paper towel was placed on the surface of the water to collect weevils as they walked across. Buckets were

monitored for up to 1 h. Weevils were transported back to the laboratory, identified and placed into a ventilated container with soil. The containers were held at 10°C, with a 10:14 light:dark cycle, for a minimum of 1 month prior to use in bioassays.

Adults were collected from fairways as they emerged from overwintering sites on 24 April 2007 from Upper Montclair Country Club (Clifton, NJ) between 0600 and 0800 h. Adults were removed using a vacuum/leaf blower (Homelite Vac Attack II, Anderson, SC) with a modified intake to capture weevils before entering the engine (Rothwell 2003). The intake was fitted with a basket with 324-mesh (7.2 × 7.2 per cm^2) bottom to allow for air to enter and keep a constant suction. After vacuuming, the basket was removed and adults were emptied into a polyethylene bag for transport back to the laboratory. In the laboratory, adults were stored at 10°C, with a 10:14 light:dark cycle until they were used in assays (< 1 wk).

Since rearing of immature stages in the laboratory has been difficult, *L. maculicollis* field infested turf cores were used to test nematode virulence to late instars. Cores (5.5 cm diameter × 5 cm depth) were removed each week from *L. maculicollis* infested golf course fairways at Pine Brook Golf Course in 2006 and Brooklake Country Club (Florham, NJ) in 2007 using a soil corer (Turf Tec International, Oakland Park, FL). Extensive sampling of populations was conducted in the weeks prior to core removal to predict fourth and fifth instar peaks. Cores were removed on dates when the estimated stage for testing was predicted to be in greatest density. Instars were confirmed in a subset of cores by extracting weevils manually and measuring head capsules according to Cameron and Johnson (1971).

Adult assays

Two separate experiments were conducted on overwintering and spring-emerged adults, respectively. Bioassays were conducted in 24-well plates (1.9 cm^2 per well). Ten wells were used on each plate and spaced as to limit the possible movement of nematodes between wells. Each cell received 2.58 g soil (61% sand, 27% silt, 12% clay, 2.3% organic matter, pH 5.9) that had been pasteurized (3 h at 70^{o}C) and air-dried before use. Then nematodes were added to the wells in 0.42 ml of water to bring the total weight of the sample to 3 g (14% moisture w/w = -6 kPa soil water potential). Adult *L. maculicollis* were brought to room temperature 1 h prior to placement into wells. Adults were placed singly into wells along with pieces of *P. annua* leaves. To confine adults to their wells, a layer of Parafilm was fitted across the top of the plate, lidded and held in place by rubber bands. Plates were then placed into a large plastic box (12 × 20 × 32 cm) with moistened paper towels to ensure high humidity (85–95%) throughout the experiment. The boxes were placed into a 20°C incubator with a light cycle of 16:8 (light:dark). The bioassay was checked every 2 d to assess mortality and to replenish *P. annua* leaves. Dead adults were dissected to determine infection by nematodes. Mortality was assessed by comparing the number of live weevil stages to the number of live stages in the control.

In the first and second experiment, overwintering and spring-emerged adults, respectively, were exposed to a high (250 IJs/well) or a low concentration (50 IJs/well) of each nematode species/strain or an untreated control. In a third experiment, overwintering adults were exposed to 0, 10, 20, 40, 80, 120 and 200 IJs/well of *S.*

carpocapsae PB to determine the concentration response. Each experiment was repeated twice with 20 replicates per treatment per repetition.

Larval assays

Infested turf cores were placed into 120-ml plastic cups (6 cm diameter) with perforated bottoms. Pasteurized sandy loam soil was used to fill any gaps between the cores and the cup's wall. Cups were placed on plastic trays by treatment, and water was applied to the bottom of the tray to ensure adequate soil moisture throughout the experiment. Each treatment consisted of 15 replicates, organized in three replicates of five cores each. Concentrations consisted of either 0, 187 IJs ($8.3/cm^2$) or 563 IJs per core ($25/cm^2$). The experiments were held at 20°C with a light cycle of 16:8 (light:dark). After 14 d, cores were inspected for *L. maculicollis* stages by hand, and then the soil and plant material was submerged in a saturated salt solution in warm water to extract unseen individuals. Mortality was assessed by comparing the number of live weevil stages to the number of live stages in the control. One assay per instar was conducted in each year.

Statistical analyses

Statistical analyses were conducted with Statistix 8.0 (Tallahassee, FL). Daily and cumulative adult percent mortality data were arcsine transformed before one-way analysis of variance (ANOVA). Where significant differences were detected, pairwise comparisons were made using Tukey's mean separation test at $\alpha = 0.05$ level. The effect of overwintering condition on the susceptibility of adults to nematodes was analyzed by Mann-Whitney non-parametric t tests, and if no significant differences were found, the data were combined. Cumulative mortality by nematode treatment was control-corrected using Schneider-Orelli's formula (Püntener 1981) since > 10% control mortality occurred

in several untreated check replicates. Probit analysis (SAS Institute 1996) was used to generate LC_{50} values, 95% fiducial limits and slope for adult mortality in dose response assays with *S. carpocapsae* (PB).

Listronotus maculicollis larval counts in five cores (one block) were square-root transformed before performing analyses. All data were combined and analyzed by one-way ANOVA to determine if nematode treatment, concentration, instar and year had significant effects on *L. maculicollis* density. Interactions between factors (nematode species and concentration, nematode species and instar tested) were analyzed by two-way ANOVA. The effect of nematode treatment on *L. maculicollis* densities in cores was represented as reduction relative to the control in addition to means separation by Tukey's test.

Results

Adult Bioassays

Dissection of the dead adults proved to be an unreliable means to confirm nematode infection. No nematodes were found in dissections and only few nematodes emerged from groups of cadavers within the same treatment placed on emergence traps. Therefore, the assessment of nematode efficacy had to be based on mortality relative to the untreated adults.

Overwintering state had no significant effect on nematode-susceptibility of adult *L. maculicollis*, and therefore the data were combined. Significant adult mortality (F = 4.33; df = 8, 65; $P < 0.0001$) was observed for the high rates of endemic *S. carpocapsae,* the commercial strain of *H. bacteriophora* and the combination of the endemic strains of

H. bacteriophora and *S. carpocapsae* (Fig. 3.1). Mortality rates did not differ significantly between endemic and commercial strains/isolates of *H. bacteriophora* and *S. carpocapsae*. Mortality from the combination treatment was not significantly different from the high rate of either species alone. Only the combination of the endemic *H. bacteriophora* and *S. carpocapsae* caused significant mortality at 50 IJs/well ($F = 2.13$; df = 8, 65; $P < 0.048$) when analyzed separately by concentration. No differences were detected between the high and low rates of nematode treatments, although numerically higher mortality was observed in all treatments except *S. kraussei.* But even the high rates only caused moderate levels of control (50–60%). Furthermore, in all treatments mortality increased gradually with even the top performing species taking 6 d to cause > 50% mortality (Fig. 3.2). Further dose response assays with *S. carpocapsae* confirmed this, generating a relatively high LC_{50} of 125 IJs per adult (95% FL: 90–204, slope = 1.95).

Larval bioassays

Commercial and endemic nematodes caused significant reductions of fourth and fifth instars in the naturally infested cores (Table 3.2). Significant differences were observed in *L. maculicollis* counts by nematode species/strain, instar and year, but no significant effect of concentration. Data were therefore combined by nematode treatment but analyzed separately by year.

In 2006, all treatments resulted in significantly lower densities of fourth instars than in the controls ($F = 8.09$; df = 5, 32; $P = 0.0001$), but there were no differences among species (67–95% control-corrected reduction). Untreated check densities were 77.4/0.09 m^2 (Table 3.3). Greater densities in 2007 (104.0/0.09 m^2) allowed for greater

separation of species ($F = 4.81$; df = 7, 44; $P < 0.0006$), but there was more variability in reduction levels (37–92% control-corrected). *Steinernema feltiae* Filipjev and *S. carpocapsae* BU caused the highest reductions and but were significantly different only from the commercial strain of *H. bacteriophora* and the control. *Steinernema kraussei* was the only other species that caused significant reduction. No differences were observed between commercial and endemic strains of *H. bacteriophora* and *S. carpocapsae*.

Fifth-instar densities in 2006 were low (24.0/0.09 m^2) and no differences among treatments could be observed. In 2007, fifth instar densities were high (165.0/0.09 m^2) and all treatments except *H. megidis* Poinar and *H. bacteriophora* PB caused significant reductions ($F = 6.89$; df = 7, 44; $P < 0.0001$). *Steinernema feltiae* caused 84% reduction compared to the control and was the only species that reduced the extreme densities below what is considered to be a damaging threshold in the field (325/m^2) (Table 3.3). However, *S. feltiae* reductions were not significantly different from *S. carpocapsae* PB (75%), *S. kraussei* (72%), *S. carpocapsae* BU (69%) or *H. bacteriophora* BU (68%). The hierarchical trend in the level of reduction by species was identical for the top four strains in the 2007 fourth-instar assays, with all four being *Steinernema* species. No differences were found between commercial and endemic strains of *H. bacteriophora* and *S. carpocapsae*.

Larval densities in the controls did not differ significantly between fourth and fifth instars within year, thus allowing for between stages comparisons of nematode virulence. In 2006, densities were significantly affected by nematode treatment ($F = 3.19$; df = 4, 59; $P = 0.02$) but not by concentration or instar and there were no significant

interactions between nematode treatment and concentration or nematode treatment and instar. *Steinernema kraussei* performed significantly poorer than the other nematodes. In 2007, densities were significantly affected by nematode treatment ($F = 7.39$; df = 6, 83; $P < 0.0001$) and instar ($F = 27.74$; df = 1, 83; $P < 0.0001$) but there were no significant interactions between nematode treatment and instar. Significantly lower *L. maculicollis* counts were found in nematode treatments against the fourth instars. *Steinernema feltiae* performed significantly better than the heterorhabditids but not statistically different from the other steinernematids in fourth and fifth instar combined bioassays.

Discussion

The results from laboratory assays strongly suggest that nematodes, at least currently available species/strains, are not sufficiently virulent to *L. maculicollis* adults to be a viable option for replacing synthetic chemical insecticides in a preventive management program on any golf course. However, applying nematodes curatively against *L. maculicollis* larvae as they enter the soil appears to have great promise. Several nematode species were able to cause significant mortality to *L. maculicollis* larvae in naturally infested turf/soil plugs under controlled conditions. These species should be further tested under field conditions to delineate their potential for incorporation into a biological control program. In particular, *S. feltiae* and *S. carpocapsae* caused consistently high levels of reduction of fourth- and fifth- instar *L. maculicollis*. Nematode isolates from locally nematode-killed *L. maculicollis* were not more virulent than the commercial species to any stage tested.

This study provides evidence for decreased nematode susceptibility with *L. maculicollis* development. Fourth- and fifth- instar larvae were susceptible to nematodes at standard field rates, with most nematode species causing significant reductions, especially *S. feltiae* and *S. carpocapsae* which caused > 90% reduction of fourth- instar densities. The nematode susceptibility of fourth- instar *L. maculicollis* was significantly greater than that of fifth instars. Many studies have demonstrated a decrease in larval susceptibility to nematodes with development (Kaya 1985; Glazer and Navon 1990; Medeiros et al. 2000; Koppenhöfer and Fuzy 2004) with several examples within the family Curculionidae (Bélair and Boivin 1985; Boivin and Bélair 1989; Shapiro et al. 1999; Shapiro-Ilan 2001; Cabanillas 2003). The carrot weevil [*Listronotus oregonensis* (Le Conte)], a close relative of *L. maculicollis* is more susceptible to *S. carpocapsae, S. feltiae* and *H. bacteriophora* late in larval development than as a pupa or adult (Boivin and Bélair 1989). But examples for the contrary also exist within Curculionidae (Schroeder et al. 1994; Loya and Hower 2003) suggesting that the relationship between age and susceptibility is species specific.

In contrast to larval susceptibility, adult mortality was only moderate when tested at five times higher rates suggesting that adults are considerably less nematode susceptible than larvae. The mechanism underlying this effect is unclear at the present, but could be behavioral (i.e., grooming by adults) or physical (i.e., differences in their exoskeleton or the structure of the mouth, anal, or spiracular openings). Overwintering adults would have been an ideal target since they are initially aggregated upon emergence (B. McGraw unpublished data) allowing for nematode applications to be concentrated in space. Unfortunately, an improvement over the low susceptibility to nematodes even

after extended periods of overwintering was not observed. *L. oregonensis* adults have been shown to be less susceptible to nematodes after overwintering than when newly formed (Boivin and Bélair 1989). However, the time to infect 50% of the population (LT_{50}) was observed to decrease when food sources were made available. If the same effect holds true with *L. maculicollis*, adult mortality might have been even lower had fresh food sources not been continuously provided.

I was unable to detect nematodes following dissection of adult cadavers, and only few nematodes emerged from groups of cadavers placed on emergence traps suggesting that adult *L. maculicollis* are poor hosts for entomopathogenic nematodes. In the Colorado potato beetle, *Leptinotarsa decemlineata*, the nematode *H. marelatus* is capable of penetrating and releasing its symbiotic bacterium (Armer et al. 2004), but enteric bacteria interfere with growth of the nematode's s symbiotic bacterium and completion of the nematode's reproductive cycle (Blackburn et al. 2007). A similar interference could be responsible for the lack of nematode recovery and/or reproduction in *L. maculicollis*

Endemic *H. bacteriophora* and *S. carpocapsae* isolates from *L. maculicollis* cadavers were not more virulent than commercial strains against any stage tested. Several authors have suggested that fresh field isolates of nematodes might perform better against local pests since they have been naturally selected for the environment or may be more specific to the insect it was reared from (Bedding et al. 1993; Iraki et al. 2000; Millar and Barbercheck 2001; Ehlers et al. 2005; Morton and García del Pino 2008). Endemic nematodes have performed significantly better than other species in numerous laboratory and field studies targeting weevil species, including pine weevil [*Hylobius abietis* (L.)] (Dillon et al. 2006; Torr et al. 2007), guava weevil [*Conotrachelus*

psidii (L.)] (Dolinski et al. 2006), alfalfa snout weevil [*Otiorhynchus ligustici* (L.)] (Schroeder et al. 1994) and clover root curculio [*Sitona hespidulus* (F.)] (Loya and Hower 2003). However, in field studies the improved efficacy of endemic strains may be in part due to better persistence (Schroeder et al. 1994; Loya and Hower 2003). Thus, field isolates collected during field surveys for natural enemies and pathogens of the pecan weevil [*Curculio caryae* (Horn)] failed to improve (Shapiro-Ilan et al. 2003) upon the low control of *C. caryae* reported in earlier laboratory studies with standard strains (Shapiro-Ilan 2001).

Nematodes are relatively immobile and therefore susceptible to genetic isolation (Liu et al. 2000) especially when populations reach low densities. The underlying genetic mechanisms that allowed for endemic species to persist in *L. maculicollis* infested areas may not have been selected for specificity with *L. maculicollis,* a spatially and temporally patchy host (McGraw and Koppenhöfer 2008a). Nematodes are likely to avoid localized extinctions by adopting a generalist strategy. Attacking a diversity of hosts would be particularly advantageous in environments where insect hosts are seasonally abundant. Although the field isolates did not show significant improvement over the commercial counterparts, this does not exclude the possibility that new species or other new field isolates could be more virulent to different *L. maculicollis* stages.

I opted to evaluate fourth and fifth instars in field-infested cores for virulence testing because no effective rearing method for *L. maculicollis* has been developed to date. The larval stages tested represent the most appropriate stages to target in field applications, even though field surveys have indicated that all stages between third instar and pupa can become infected by nematodes (McGraw and Koppenhöfer 2008a). I did

not test third instars and pupae since nematodes are not likely to be in close contact with the stem-boring third instars, and targeting pupae will not reduce turfgrass damage and might, at best, provide some reduction of the next weevil generation. Using field-infested cores, although inherently variable in weevil density, may be advantageous by allowing different foraging behaviors of nematode species to play a role, especially with respect to infecting potentially cryptic larvae. *Heterorhabditis bacteriophora* is a "cruiser" that actively seeks out its prey in the soil and has the potential advantage of finding sedentary larvae (Campbell and Gaugler 1997; Lewis et al. 2006). *Steinernema carpocapsae* employs an ambush strategy (Campbell and Gaugler 1993), awaiting its prey at the soil surface, and usually outperforms most other species in Petri dish assays conducted in shallow soil. Conducting the assays in a realistically complex environment reduces the likelihood of producing this kind of laboratory artifact. *Steinernema feltiae* showed the greatest and most consistent control of larvae, which could be due in part to its intermediate foraging strategy (Lewis et al. 2006), ambushing larvae as they move between plants and cruising after more sedentary late instar larvae in the soil. Since *L. maculicollis* utilize different habitats as they age and can often be in-between habitats for considerable periods, a nematode with a flexible foraging strategy could prove to be most effective. The fact that the top performing nematode treatment against adults was the combinations of *S. carpocapsae* and *H. bacteriophora* provides further evidence for greater control by combining foraging strategies, albeit using two nematode species.

Nematodes are virulent to numerous weevil species and have also been successfully implemented in the management of some turfgrass insect pests (Parkman et al. 1996; Klein 1993; Georgis and Poinar 1994). However, the integration of nematodes

into a management program for *L. maculicollis* has many barriers to overcome. Given the significant differences in the susceptibility between fourth and fifth instars, the rate and timing of application are likely to be major factors in the control of *L. maculicollis*. Young instars are stem borers and unlikely to come into contact with nematodes. If applications are made too soon, nematode numbers may already have declined below effective levels by the time the larvae emerge into the soil. Field studies will need to address how long the nematodes can persist in the soil and, with that, how precisely applications need to be timed with pest phenology so that high numbers of nematodes are present when larvae start entering the soil. *L. maculicollis* densities are temporally and spatially variable and finding an application rate ensuring coverage across the entire period that larval stages are entering the soil and all possible density scenarios might prove difficult. The second and third generations are more uniform, less dense and typically cause less damage than the first generation. However, the lower densities may warrant intervention if damage from larval feeding is exacerbated by other environmental stresses such as high temperatures and low soil moistures. Field trials in New Jersey targeting these summer generations have been thus far unsuccessful in part to the less than optimal environmental conditions for nematode infection and persistence following application (B. McGraw, unpublished data).

Variable control as observed in early field trials is likely to occur in the future given the inherent variability of the pest's phenology and the sensitivity of nematodes to environmental conditions. It is widely acknowledged that great variability can also exist in virulence between nematode strains. The high aesthetic expectations in golf course turfgrass management leave little room for variability in pest management. Considering

the present costs of biological control agents in comparison to conventional chemical pesticides, lower control will not be tolerated in many situations. Successful implementation of biological control programs on golf courses will require a greater understanding of the ecology of entomopathogenic nematodes and how to favorably manipulate the system into which they are applied.

Table 3.1. Entomopathogenic nematode strains used in assays and abbreviations and sources thereof.

Species	Strain	Source	Abbreviation	Assays[a]
Steinernema carpocapsae[b]	BU	Becker Underwood	SC (BU)	OW, E, L 06, L 07
S. carpocapsae[c]	PB	Pine Brook G.C., Manalapan, NJ	SC (PB)	OW, E, L 07
S. feltiae[b]	BU	Becker Underwood	SF	OW, E, L 06, L 07
S. kraussei[c]	CA	Guy Bélair, Agri-Canada	SK (CA)	OW, E
S. kraussei[b]	BU	Becker Underwood	SK (BU)	L 06, L 07
Heterorhabditis bacteriophora[b]	BU	Becker Underwood	HB (BU)	OW, E, L 07
H. bacteriophora[c]	PB	Pine Brook G.C., Manalapan, NJ	HB (PB)	OW, E, L 06, L07
H. megidis[b]	BU	Becker Underwood	HM	OW, E, L 06, L 07
S. carpocapsae[c] + *H. bacteriophora*[c]	PB	Pine Brook G.C., Manalapan, NJ	HB-SC (PB)	OW, E, L 06, L 07

[a] Assays: OW = overwintered adult, E = spring-emerged adult, L= fourth and fifth instar, 06 = 2006, 07 = 2007

[b] Commercially produced in vitro

[c] Produced in vivo in the laboratory

Table 3.2. Analysis of Variance (ANOVA) table for 2006, 2007, and combined bioassays.

	FACTOR	DF	MS	F	P
All Assays	SPECIES	7	4.46	8.01	< 0.0001
	INSTAR	1	7.30	13.20	0.0004
	YEAR	1	27.9	50.10	< 0.0001
	CONCENTRATION	1	1.57	2.77	0.1
2006	SPECIES	4	0.74	3.19	0.02
	CONCENTRATION	1	0.27	1.17	0.29
	INSTAR	1	0.07	0.34	0.56
	SPECIES * CONCENTRATION	4	0.21	0.94	0.45
	SPECIES * INSTAR	4	0.21	0.90	0.47
2007	SPECIES	6	3.55	7.39	< 0.0001
	CONCENTRATION	1	1.14	2.37	0.13
	INSTAR	1	13.34	27.74	< 0.0001
	SPECIES * CONCENTRATION	6	0.52	1.08	0.39
	SPECIES * INSTAR	6	0.99	2.05	0.07

Table 3.3. Effect of exposure to entomopathogenic nematodes on density (number per five cores) of fourth- and fifth-instar *Listronotus maculicollis* in laboratory bioassays in 2 years (2006, 2007). Data shown are combination of a high (25 infective juveniles/cm^2) and low (8.3/cm^2) concentration. Percent reduction is expressed in density relative to the density in the untreated control. Different letters within year and instar indicate significant differences (Tukey's pairwise comparison test, $P < 0.05$)

		2006		2007	
Stage	**Species**	**Larvae (± SE)**	**CCR***	**Larvae (± SE)**	**CCR**
Fourth Instar	*S. feltiae*	1.3 ± 0.4 b	86%	1.0 ± 0.8 c	92%
	S. carpocapsae PB	n/a		1.7 ± 0.8 c	87%
	S. kraussei	3.2 ± 0.6 b	67%	3.2 ± 1.0 bc	76%
	S. carpocapsae BU	0.5 ± 0.3 b	95%	4.2 ± 1.7 abc	68%
	H. bacteriophora PB	1.8 ± 1.3 b	81%	4.3 ± 1.7 abc	67%
	H. megidis	2.3 ± 0.4 b	76%	5.0 ± 1.4 abc	62%
	H. bacteriophora BU	n/a		8.2 ± 1.6 ab	37%
	Control	9.7 ± 1.9 a		13.0 ± 3.8 a	
Fifth Instar	*S. feltiae*	1.8 ± 0.4 a	39%	3.3 ± 1.2 d	84%
	S. carpocapsae PB	n/a		5.2 ± 0.8 cd	75%
	S. kraussei	2.5 ± 0.4 a	17%	5.8 ± 0.1 bcd	72%
	S. carpocapsae BU	1.7 ± 0.6 a	44%	6.5 ± 1.7 bcd	69%
	H. bacteriophora BU	n/a		6.7 ± 0.9 bcd	68%
	H. bacteriophora PB	1.3 ± 0.5 a	56%	11.6 ± 1.6 abc	44%
	H. megidis	2.0 ± 0.7 a	33%	13.0 ± 1.4 ab	37%
	Control	3.0 ± 2.0 a		20.7 ± 7.5 a	

*CCR= Control Corrected Reduction

Figure 3.1. Cumulative mortality of *Listronotus maculicollis* adults after 12 d of exposure to 50 or 250 infective juveniles (IJs) of different entomopathogenic nematodes species/strains in the laboratory. Means with same letters over bars (within concentration category) are not significantly different (Tukey's pairwise comparison, $P < 0.05$). Sf (BU) = *Steinernema felitae* BU strain; Sk (CA) = *S. kraussei* CA strain; Hm (BU) = *Heterorhabditis megidis* BU strain; Sc (BU) = *S. carpocapsae* BU strain; Hb (PB) = *H. bacteriophora* PB strain; Sc (PB) = *S. carpocapsae* PB strain; Hb (BU) = *H. bacteriophora* BU strain; Hb-Sc (PB) = *H. bacteriophora* PB strain combined with *S. carpocapsae* PB strain.

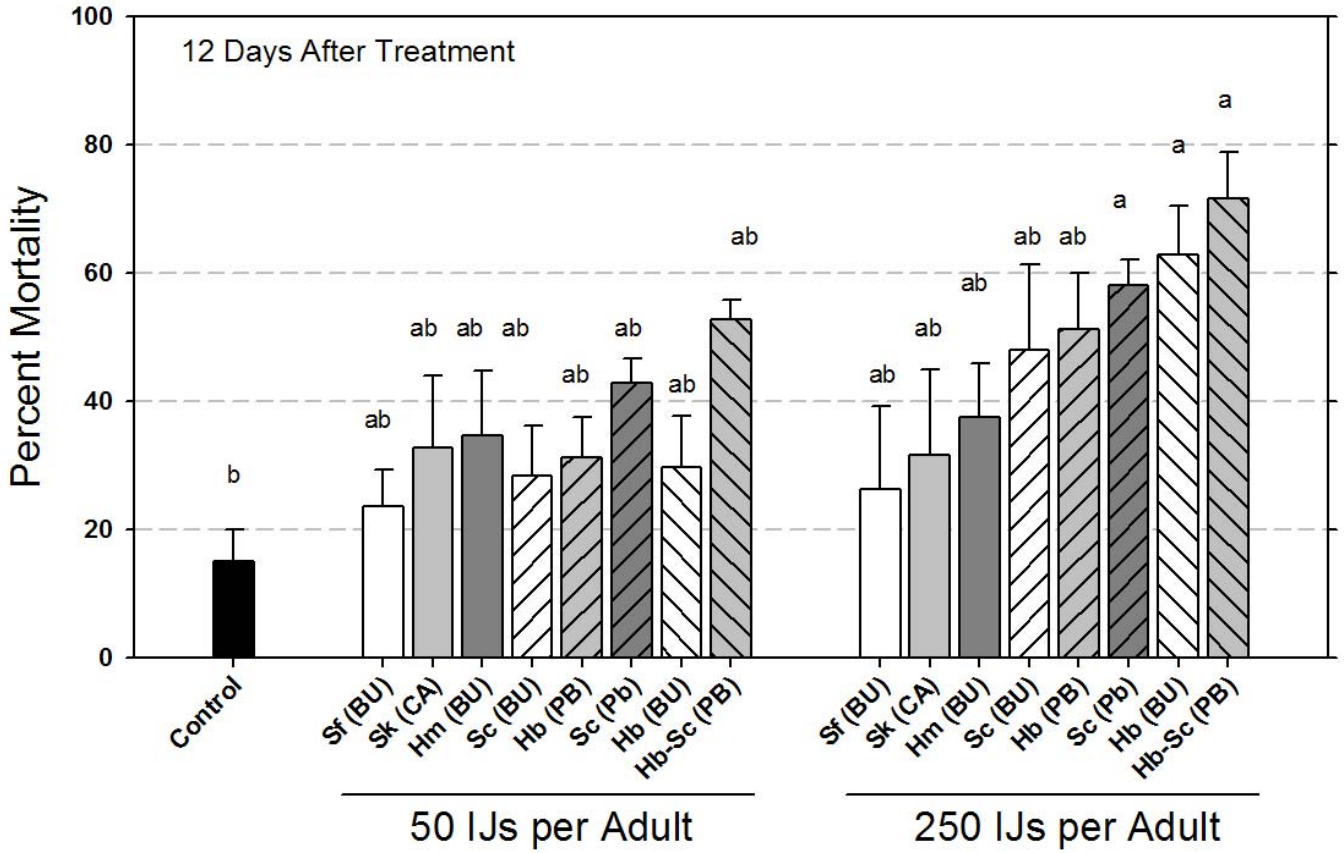
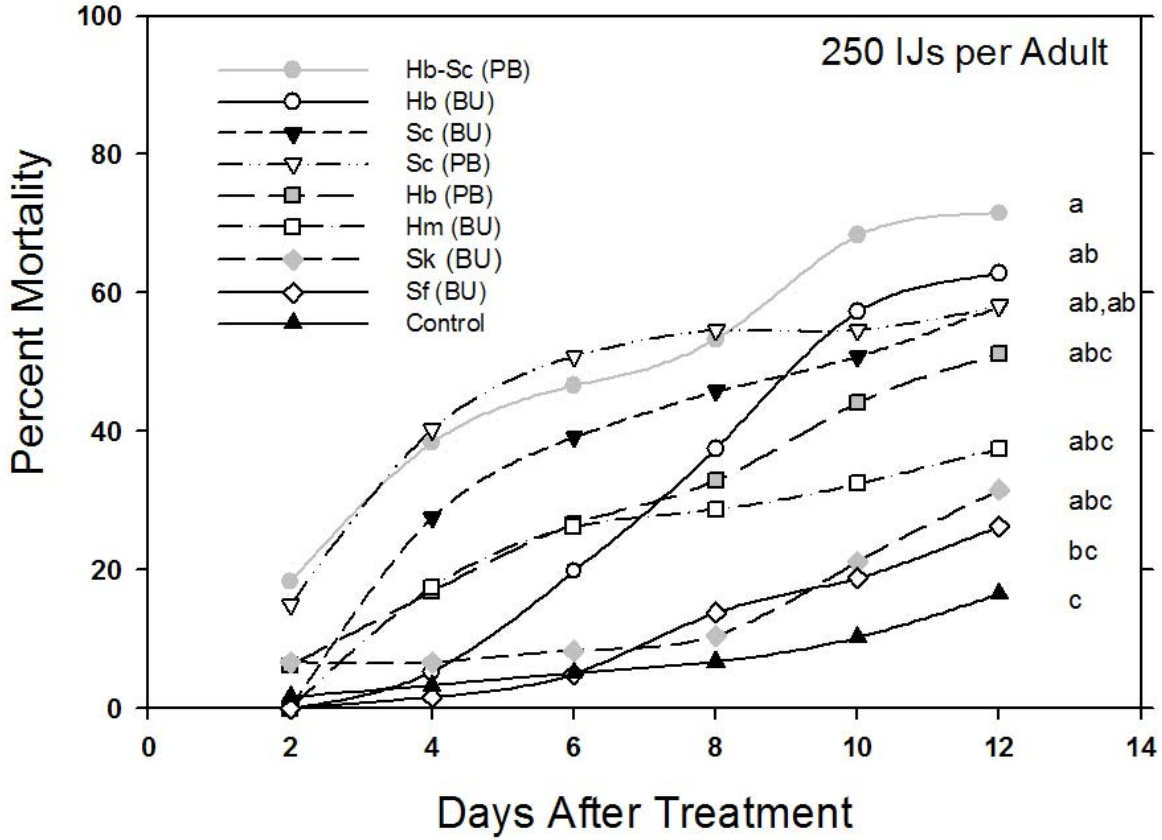


Figure 3.2. Effect of length of exposure on mortality of *Listronotus maculicollis* adults exposed to 250 infective juveniles (IJs) per adult in the laboratory. Letters indicate significant differences among means at 12 d after treatment. Hb-Sc (PB) = *Heterorhabditis bacteriophora* PB strain combined with *Steinernema carpocapsae* PB strain; Hb (BU) = *H. bacteriophora* BU strain; Sc (BU) = *S. carpocapsae* BU strain; Sc (PB) = *S. carpocapsae* PB strain; Hb (PB) = *H. bacteriophora* PB strain; Hm (BU) = *H. megidis* BU strain; Sk (BU) = *S. kraussei* BU strain; Sf (BU) = *S. felitae* BU strain.



CHAPTER FOUR

FIELD EVALUATION OF ENTOMOPATHOGENIC NEMATODES FOR THE BIOLOGICAL CONTROL OF THE ANNUAL BLUEGRASS WEEVIL, *LISTRONOTUS MACULICOLLIS* (COLEOPTERA: CURCULIONIDAE) IN GOLF COURSE TURFGRASS

Abstract

The potential of commercial and endemic entomopathogenic nematodes to control larval populations of *L. maculicollis*, a highly destructive pest of short mown turfgrasses was evaluated under field conditions over a three year period (2006–2008). Single inundative releases of *Steinernema carpocapsae* Weiser, *S. feltiae* Filipjev, and *Heterorhabditis bacteriophora* Poinar applied in concentrations of 2.5×10^9 IJs/ha reduced first generation late instar larvae between 69 and 94% in at least one field trial. *Steinernema feltiae* BU provided a high level of control (94%) to low densities (approximately 20 larvae per $0.09m^2$) in the first trial, though failed to provide adequate control at higher larval densities in two additional trials (24 and 50% control). *Steinernema carpocapsae* BU provided the most consistent control of *L. maculicollis* (63–69%), though never exceeded 70% control with standard field rates. Multiple species releases and split rate applications applied in two consecutive weekly treatments provided numerical, but not statistical improvement of the level of control with single

species applied once at a rate of 2.5×10^9 IJs/ha. Nematode persistence was generally low in all treatments and experiments. Nematode densities, based on number of nematode-infected bait insects, strongly declined between 0 and 14 d. An increase in the number of nematode-infected baits was observed 28 d after application in most treatments, suggesting that entomopathogenic nematodes are capable of recycling in the golf course environment. However, applied nematodes were practically undetectable 56 d after application, when second generation larvae were present in the soil. This study suggest that single inoculative applications of currently commercially available species of entomopathogenic nematodes applied at standard field rates cannot reliably reduce *L. maculicollis* larval densities on golf courses and that they are unlikely to have a significant effect on later pest generations. Additional research will be required to identify application strategies to improve upon the inconsistent control observed in the field.

Introduction

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is a serious pest of short mown golf course turfgrass and other well irrigated turf stands in the northeastern United States and eastern Canada (Vittum et al. 1999; Simard et al. 2007). Feeding by populations of late instar larvae can cause substantial damage to turf, killing areas measuring from tens to hundreds of square meters of turf if left unchecked. The potential for low densities of larvae to cause damage (B. McGraw unpublished data) and low aesthetic thresholds for visible turfgrass damage have caused turfgrass managers to adopt primarily preventive chemical control strategies for managing *L. maculicollis*

populations. Multiple chemical insecticide applications are typically made against the two or three generations of *L. maculicollis* that occur throughout the region (McNeil et al. 1999), creating a strong selection pressure for the development of insecticide resistant populations. Consequently, insecticide resistance has become a reality in areas throughout the weevil's distribution (Cowles et al. 2008).

Adult *L. maculicollis* spend late Fall through early Spring in overwintering sites, in tall grasses and leaf litter adjacent to short mown areas (Diaz and Peck 2007). As temperatures warm in spring, adults emerge from overwintering sites, walk on to the close cut playing surfaces, feed and mate (Rothwell 2003). Management of *L. maculicollis* is usually conducted through chemical means, focusing primarily on avoiding larval feeding damage by controlling overwintering adults moving between overwintering sites and short mown playing surfaces. Though the adults may chew notches in the stem of the plant, the majority of damage to the turf comes from larval feeding. *Listronotus maculicollis* go through five larval instars, the first three of which are spent above the crown inside the plant, largely protected from most chemical controls. Fourth and fifth instars feed externally on the crown, ultimately severing the actively growing portion of the plant. Later instar larvae can be found moving between the thatch and soil interface as they feed, eventually moving lower in the soil profile (3–5 cm) to form an earthen cell to pupate. In New Jersey, feeding damage from first generation late instar larvae is typically visible between the first and third week of June. Two more generations complete development between June and October, though population densities are lower and typically damage is less common (McGraw and Koppenhöfer 2008a).

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (Rhabditida) are obligate, generalist pathogens of insects, present in soils of many ecosystems around the world (Adams et al. 2006). These nematodes spend a portion of their life cycle as a free-living, non-feeding injective juvenile (IJ), which enters its host through natural openings (mouth, spiracles, anus) or, in some cases, directly through the cuticle (Bedding and Molyneux 1982). Once inside the insect's hemocoel, IJs release a symbiotic bacterium that kills the host through septicemia (Kaya and Gaugler 1993). The bacteria also convert the host's tissues to suitable substrate for nematode development and reproduction. After the nematodes have developed through one to three generations and resources in the cadaver are depleted, hundreds to hundreds of thousands of IJs emerge to seek out new hosts.

Entomopathogenic nematodes have long been employed as inundative biological control agents for soil dwelling pests (Kaya and Gaugler 1993). They possess many positive qualities needed by a biological control agent such as the ability to seek out hosts through different foraging strategies (Grewal et al. 1994b), cause rapid death, produce potentially tens to hundreds of thousands of progeny from a single infection, and recycle through alternate hosts when pests are scarce (Kaya 1990). However, nematode sensitivity to extreme temperature, UV light and low moisture may limit their efficacy and persistence in some environments. Golf course turfgrass provides an environment that may be manipulated to improve entomopathogenic nematode efficacy against target pests and persistence during periods with limited hosts. Additionally, entomopathogenic nematodes have been shown to be tolerant of a wide range of chemicals and amendments commonly used in turfgrass management (Krishnayya and Grewal 2002; Koppenhöfer

and Grewal 2005; Alumai et al. 2006) and are capable of being applied with standard golf course equipment or in combination with chemical pesticides. In some instances, nematodes have been documented to interact synergistically with synthetic insecticides used on golf courses (Koppenhöfer and Kaya 1997).

Listronotus maculicollis have been identified as a potential target for biological control using entomopathogenic nematodes (Georgis et al. 2006), since they spend most of their life cycle and a significant portion of the year in the soil or on the soil surface. Three independent field studies have detected nematodes infecting *L. maculicollis* immature stages, which to date are the weevil's only described natural enemies (Vittum 1980; Grant and Rossi 2004; McGraw and Koppenhöfer 2007, 2008a). McGraw and Koppenhöfer (2008a) detected epizootics in weevil populations on three courses in New Jersey and documented the impact that endemic populations have on weevil populations. Laboratory bioassays using commercial nematode products and *Steinernema carpocapsae* Weiser and *Heterorhabditis bacteriophora* Poinar strains isolated from *L. maculicollis* suggest that adults are not highly susceptible to nematode infection (McGraw and Koppenhöfer 2008b). However, high levels of control were observed when nematodes were applied at standard field concentrations (2.5×10^9 IJ/ha) to fourth and fifth instar larvae.

The objectives of this study were to compare the relative virulence of commercial and endemic species/strains of entomopathogenic nematodes to first generation *L. maculicollis* larvae under field conditions and to identify the best candidates for augmentative biological control programs. Secondly, I sought to determine the relative benefit of multiple-species and split-concentration applications when compared to single-

species applied once. Finally, I documented the persistence of various species and concentrations thereof to determine the potential for inoculative releases to recycle in the environment and control multiple *L. maculicollis* generation. The findings presented here should aid in the development of biological control programs using entomopathogenic nematodes to control *L. maculicollis*.

Materials and Methods

Nematodes

Five commercially available nematodes species (*Steinernema carpocapsae*, *S. feltiae* Filipjev, *S. kraussei* Filipjev, *Heterorhabditis bacteriophora* and *H. megidis* Poinar BU strains) were obtained from Becker Underwood (Sussex, U.K.) for use in field trials. Formulated nematode products were delivered to the Turfgrass Entomology Laboratory at Rutgers University in cooled shipping containers. Nematodes were placed into an unlit 10°C incubator upon arrival. An endemic nematode, *H. bacteriophora* (PB isolate) was isolated from infected *L. maculicollis* cadavers at Pine Brook Golf Course in Manalapan, NJ in June 2005.

Field sites and design

Field trials were conducted over a three year period (2006–2008) at Pine Brook Golf Course (PB), Upper Montclair Country Club (UMCC) (Clifton, NJ) and Brooklake Country Club (BLCC) (Florham Park, NJ). Treatments were applied in a randomized complete block design, replicated six times per trial. Treatment replicates were halved between blocks either on different fairways of the same golf course (PB in 2006, BLCC and UMCC in 2008), or on different golf courses (UMCC and BLCC in 2007).

Treatments were applied to plots measuring 1.52 m (length along rough-fairway border) by 1.83 m (width into the fairway) (2.78 m^2). Plots were separated by 30 cm on each side to limit nematode movement into the neighboring plots. Previous research demonstrated an edge bias to the distribution of *L. maculicollis* larvae (B. McGraw unpublished data). To reduce variability of larval densities between replicates, blocks were arranged parallel to the edge of the rough-fairway border with a maximum width of two replicates (3.96 m).

Field application of entomopathogenic nematodes

The concentration of nematodes in formulated products were estimated 12 h prior to application by diluting subsamples of the product in water, and calculating the number of live nematodes in 16 10 µl droplets. The value was then used to calculate the number of live nematodes per gram of formulated product. Nematode treatments were weighed, placed into individual labeled 30-ml plastic cups (SOLO, Highland Park, IL) and placed into a darkened 10°C incubator until the following morning. The endemic *H. bacteriophora* was reared in late instar greater wax moth, *Galleria mellonella* L., larvae at room temperature (22–25°C) approximately a month prior to the field trials. Wax moth larvae were supplied by Northern Bait (Chetek, WI), Nature's Way (Harrison, OH) and Morning Dew Bait (Trumansburg, NY). Infective juveniles were harvested as they emerged from nematode-killed insects over a 2-wk period and stored at 10°C in Petri dishes (100 × 15 mm) 1–2 wk prior to application. The concentration of the endemic isolate was estimated in the same manner described for the commercial species. The endemic IJs were stored in 225 ml tissue culture flasks at 10°C overnight in concentrations of 5,000/ml. On the day of application, all nematodes were transported to

the field in a cooler with an ice pack to maintain temperatures around 20°C until application.

Applications were timed based on estimated larval peaks in the weekly sampling from concurrent population dynamic studies (McGraw and Koppenhöfer 2008a). Applications were intended to target the larval population entering the soil (fourth instars), 1wk after the peak of third instar abundance. At the time of application, the weather and soil temperatures at 5 cm were recorded. Nematodes in formulated product or in solution were applied in watering cans with 3.1 liter of water, followed by 3.1 liter of water alone (rinse), for a total of 3.1 mm of irrigation.

Experiments 1: Nematode species and concentration

The effect of nematode species/strain and IJ concentration on the abundance of first generation *L. maculicollis* immature stages was assessed in three separate field trials (2006–2008). In the first trial, four commercial (*S. feltiae*, *S. carpocapsae*, *S. kraussei*, and *H. megidis* BU strains) and one endemic nematode isolate (*H. bacteriophora* PB) were tested at standard field rate (2.5×10^9 IJs/ha). The following experiment examined the virulence of the same nematode strains plus a commercial strain of *H. bacteriophora* at two concentrations (2.5 and 1.25×10^9 IJs/ha). In the last trial, the top commercial candidates from the previous field trials (*S. feltiae*, *S. carpocapsae*, and *H. bacteriophora* BU strains) were evaluated at three concentrations (5, 2.5, and 1.25×10^9 IJs/ha).

Experiment 2: Split applications of nematodes

In the second experiment, the effect of splitting nematode treatments into two half rates (1.25×10^9 IJs/ha) applied in consecutive weeks was compared to the control of using a full (2.5×10^9 IJs/ha) or half (1.25×10^9 IJs/ha) rate applied only once. The first

application of the split or repeat application, along with the full or half rate was timed to target the larval population entering the soil (fourth instars). The second application was made 1 wk later. Two species, *S. feltiae* BU and the endemic *H. bacteriophora* (PB) were compared to rates of 2.5 and 1.25×10^9 IJs/ha rates of either species applied on the first application date.

Experiment 3: Combination of entomopathogenic nematodes species

The effect of combining species of entomopathogenic nematode with different foraging behaviors to reduce the abundance of *L. maculicollis* immature stages was examined in 2007. *Steinernema carpocapsae* BU was combined with *H. bacteriophora* PB at a rate of 1.25×10^9 IJs/ha per species and compared to the 2.5×10^9 IJs/ha concentration of each species alone.

Post release monitoring

Control of *L. maculicollis* populations by entomopathogenic nematodes was assessed by comparing the densities of weevils 14 d after treatment (DAT) to densities in the untreated check. Eight soil cores were removed using a Turf-Tec international (Oakland Park, FL) turf corer (5.4 cm diameter × 5 cm depth), placed into polyethylene bags, and transported back to the laboratory in coolers. In the laboratory, samples were visually inspected for *L. maculicollis* stages by manually separating the soil from the plant material. The thatch and plant material was placed in a 500-ml beaker filled with 400 ml of a saturated salt solution in lukewarm water to irritate early instars, causing them to leave the plants. Recovered live weevil stages were incubated individually in 24-well plates on moistened filter paper at room temperature (22–25°C) for 24 h, to assess viability and allow for change in color if already infected by nematodes. Infected weevils

were placed on modified White traps (35 × 10 mm Petri dish lined with filter paper floating on tap water in a 100 × 15 mm Petri dish) (Kaya and Stock 1997) which were observed regularly to confirm cause of mortality and to harvest nematode progeny from cadavers.

Entomopathogenic nematode populations were monitored after application (0 DAT) to assess the infectivity of applied nematodes and estimate endemic entomopathogenic nematode populations in treatments. In 2006 (Experiment 1, Trial 1), the persistence of released nematodes was assessed at 0, 14 and 56 DAT. The final observation occurred during the period when second generation *L. maculicollis* larvae were beginning to enter the soil. The following year's trial included an additional observation two weeks after *L. maculicollis* evaluation (28 DAT). Nematodes released in split applications and combined species treatments were monitored 0, 14, 28 and 56 DAT.

Nematode populations were estimated by removing eight soil samples per plot using a Turf-Tec international turf corer on 0 and 14 DAT and an Oakfield sampler (1.9 cm diameter × 5 cm deep) on 28 and 56 DAT. Samples were pooled by plot in polyethylene bags and transported back to the laboratory in coolers. The pooled cores were broken up and thoroughly mixed, moistened (if necessary) to levels optimal for nematode activity (12–14% w/w), and a subsample of 75 g placed into one deep Petri dish (100 × 25 mm) per sample. Infective juveniles in soils were exhaustively baited with according to Koppenhöfer et al. (1998) with the exception of the use of five wax moth larvae per round and using 75-g soil aliquots.

Statistical analyses

Statistical analyses were conducted using Statistix 8.0 software package (Tallahassee, FL). The effect of nematode treatment on *L. maculicollis* densities was determined as the percentage reduction compared to the counts of *L. maculicollis* immature stages in the untreated plots. Adults were not included in the counts due to their ability to move between plots. Differences between treatments were analyzed by one-way analysis of variance (ANOVA). Prior to analysis, total counts of immature *L. maculicollis* per plot were square-root (x + 0.01) transformed to stabilize the variance. Two-way ANOVA was performed on the 2007 and 2008 datasets to determine if there were significant interactions between nematode species and concentration on counts of *L. maculicollis* immatures. If no differences were detected, the data were combined and the effect of nematode species on persistence was analyzed. Where significant differences were detected, pairwise comparisons were made using Tukey's mean separation test at $\alpha = 0.05$ level.

Nematode persistence in the released areas was estimated by the average number of nematode infected wax moth larvae within treatments. The number of bait insects killed was square root (x + 0.01) transformed prior to analysis by one-way ANOVA. Two-way ANOVA was performed on the 2007 trial dataset to determine if there were significant interactions between nematode species and concentration on the number of nematode infected wax moth larvae. If no differences were detected the data were combined and the effect of nematode species on persistence was analyzed. Means were separated using Tukey's mean separation test at $\alpha = 0.05$ level.

Results

Experiment 1

The effect of entomopathogenic nematode species and concentration on *L. maculicollis* populations was variable between years of the field experiment (Fig. 4.1). In the first year's trial, single concentrations of 2.5×10^9 IJs/ha generated considerable reductions of *L. maculicollis* densities (62–94%). However, pest densities across the treated area were very low and highly variable in the untreated plots, and no significant differences were found ($F = 1.77$; df = 5, 34; $P = 0.15$) when compared to the untreated controls. The lowest densities were found in the *Steinernema feltiae* BU treatment (94% lower than untreated plots), but weevil densities were well below damaging thresholds (approximately 30–40 larvae per $0.09m^2$) in all treatments.

In the following trial, *L. maculicollis* densities were numerically lower than in the untreated plots (approximately 70% control with *H. bacteriophora* BU and *S. carpocapsae* BU), but no significant differences were detected between nematodes species and the untreated controls ($F = 1.92$; df = 5, 71; $P = 0.105$) despite three-fold higher weevil densities in control plots than in the previous year. Nematode concentration did not have a significant effect on control ($F = 3.76$; df = 1, 71; $P = 0.057$), though with the exception of *S. kraussei* BU, higher concentrations improved the numerical control for each species. No significant interactions were found between species and dose ($F = 0.39$; df = 5, 71; $P = 0.855$). However, combining the doses by species did not provide significant differences in *L. maculicollis* densities among treatments ($F = 2.12$; df = 6, 77; $P = 0.062$), though *S. carpocapsae* BU neared significant improvement over *S. kraussei* BU and the untreated controls.

In the final trial the efficacy of the top commercial strains (*S. feltiae*, *S. carpocapsae*, and *H. bacteriophora* BU strains) based on performance in past field trials were examined. Despite lower larval densities than in 2007, neither the high rate (5×10^9 IJs/ha) nor the standard field rate (2.5×10^9 IJs/ha) provided control greater or even equivalent to that observed in earlier trials at 2.5×10^9 IJs/ha. *Steinernema carpocapsae* BU at 5×10^9 IJs/ha generated the largest reduction (83%), but was only significantly different from the low rate (1.25×10^9 IJs/ha) of *H. bacteriophora* ($F = 2.52$; df = 9, 59; $P = 0.018$). Since no significant dose effect was observed ($F = 0.61$; df = 2, 53; $P = 0.546$), the data were pooled by nematode species. *Steinernema carpocapsae* BU provided significantly higher control than *H. bacteriophora* BU and the untreated controls ($F = 7.54$; df = 3, 59; $P < 0.001$), but was not different from *S. feltiae* BU.

Nematode persistence in single-species inundative releases was extremely variable between sampling dates. Significant differences between nematode treatments in the number of nematode-infected bait insects were observed immediately following application (0 DAT) in both 2006 and 2007 (Fig. 4.2). In 2006, the endemic *H. bacteriophora* PB infected significantly higher numbers of bait insects than *S. feltiae* BU at 0 DAT ($F = 11.04$; df = 5, 34; $P < 0.001$), and all treatments except for *S. feltiae* BU infected significantly more bait insects after application than the untreated control. In 2007, *H. bacteriophora* PB was the only species with significantly higher numbers of infected bait insects than controls at 0 DAT ($F = 2.70$; df = 6, 77; $P = 0.02$).

Released nematodes showed limited field persistence between 0 and 56 DAT, and between 0 and 14 DAT in many treatments (Fig. 4.2). In 2006, three of the five treatments had declined ≥ 50% by 14 DAT, including the endemic strain of *H.*

bacteriophora (PB). However, *S. carpocapsae* BU and *S. feltiae* BU infections did not decrease between 0 and 14 DAT in both 2006 and 2007. Adding an extra observation period in 2007 revealed all nematode species increased between 14 and 28 DAT, with the exception of *S. feltiae* BU. However, all treatments had declined when the second generation of larvae were present in the soil (56 DAT). In contrast, the number of nematode-infected bait insects increased from 14 to 56 DAT in 2006 and 2007 and remained the same between 28 and 56 DAT in 2007. *Heterorhabditis bacteriophora*, *S. carpocapsae*, and *S. feltiae* were detected in the untreated plots after 14 days. Despite higher numbers of infected bait insects at 56 DAT in the untreated plots than in all other treatments, the number of infections were not significantly affected by treatment in 2006 ($F = 1.69$; df = 5; $P = 0.168$) and 2007 ($F = 1.47$; df = 12; $P = 0.159$).

Experiment 2

In 2007, the only treatment causing a significant reduction ($F = 3.28$; df = 6, 41; $P = 0.012$) in *L. maculicollis* densities compared to the untreated controls was the split application of *H. bacteriophora* PB applied twice at 1.25×10^9 IJs/ha (87% reduction). However, splitting applications of *H. bacteriophora* PB and *S. feltiae* BU did not improve control significantly compared to a single application of 1.25×10^9 IJs/ha or 2.5×10^9 IJs/ha. Split application did result in lower *L. maculicollis* densities compared to the single application of the full rate 2.5×10^9 IJs/ha for *H. bacteriophora* PB (87% vs. 60% lower than untreated plots) and *S. feltiae* BU (65% vs. 24%).

Nematode persistence in repeat application treatments did not improve upon the long-term dynamics of the released populations when compared to the species treatments applied at full or half rates alone (Fig. 4.4). No significant differences were detected in

bait insect mortality between treatments after application (F = 2.13; df = 6, 41; P = 0.074). In general higher densities of *H. bacteriophora* PB were found compared to *S. feltiae* BU. Although the second application of the repeated treatments were applied 7 d prior to 14 DAT, no significant differences were detected between either species and the full or half rate applied 14 d earlier (F = 0.36; df= 6, 41; P = 0.898). Repeated treatments of *H. bacteriophora* PB persisted much the same as the full rate and half rate alone, but on average infected more bait insects at 14 and 28 DAT. Endemic *Heterorhabditis bacteriophora* were detected at low population densities in the *S. feltiae* high concentration treatment and the untreated controls immediately following application. *Steinernema carpocapsae* was also detected in untreated and split application treatment plots at 14, 28 and 56 DAT. It is unclear as to whether these nematodes were from endemic populations or moved from treatments neighboring the split application experiment.

Experiment 3

Neither the multiple released species or single species treatments significantly reduced *L. maculicollis* densities compared to the untreated control (F = 0.72; df= 5, 35; P = 0.613). However, the lowest densities were observed in the combination of *H. bacteriophora* PB and *S. carpocapsae* BU (82% lower than untreated control) (Fig. 4.5).

Numbers of nematode-infected bait insects differed significant among treatments in the multi-species release experiment (Fig. 4.6). At 0 DAT, more insect baits were infected in the *H. bacteriophora* PB-*S. carpocapsae* BU combination than the at low rate of *S. carpocapsae* alone or the untreated controls (F= 5.27; df = 5, 35; P = 0.001). The high and low rates of H. bacteriophora PB were also significantly different from the

untreated controls. However, no differences in the number of infections were observed among treatments at 14, 28 and 56 DAT. In the combination treatments, greater numbers of bait insects were infected by *H. bacteriophora* PB at 0 and 56 DAT and by *S. carpocapsae* BU at 14 and 28 DAT. *Heterorhabditis bacteriophora* was detected in the untreated controls on all sampling occasions, *S. carpocapsae* on all dates between 14 and 56 DAT, and *S. feltiae* on the last sampling date. Variable numbers of *H. bacteriophora* were also found in *S. carpocapsae* treatments between 0 and 28 DAT.

Discussion

This study suggests that entomopathogenic nematodes may cause considerable reductions of *L. maculicollis* larval populations, yet control with the currently available nematode species and strains may be too variable for turfgrass managers to adopt this control option. The inherent variability in larval densities or patchiness of populations at the scale of both small plots and field trial blocks (McGraw and Koppenhöfer 2008a) likely limited the ability to detect significant effects of nematode treatments. However, some trends are discernable from the non-significant data. *Steinernema feltiae* BU and *S. carpocapsae* BU demonstrated significant control of *L. maculicollis* larvae in the laboratory (McGraw and Koppenhöfer 2008b). This was confirmed in the field trials, albeit without statistical significance. The variability in the effectiveness of entomopathogenic nematodes may be due to numerous abiotic (e.g., weather, soil characteristics, timing of applications) and biotic factors (e.g., host density, nematode natural mortality). Future research will need to address these issues if entomopathogenic nematodes are to be used effectively in *L. maculicollis* management.

In the first year of the study, one time inundative releases at standard nematode field application rates (2.5×10^9 IJs/ha) resulted in high levels of reduction (62–94%) of low larval population densities. The inability to identify top candidates was believed to be due to the low densities and variability in counts of larvae between treatment replicates. Populations of emerging, overwintering *L. maculicollis* adults walk onto short mown playing surface through the edge of the rough/fairway border (Rothwell 2003). Eggs are deposited as females first encounter short mown hosts. As the female moves across the fairway, fewer eggs are laid, creating an edge biased larval distribution (B. McGraw unpublished data). I attempted to counteract the variability in larval counts by placing treatments along the edges of fairways. In the second year of the study, the trials were moved to sites were *L. maculicollis* densities were higher. Higher densities of weevils did not improve the variability in larval the counts in either treated or untreated areas, suggesting that *L. maculicollis* larval populations are not more uniformly distributed on edges of fairways with increasing population densities.

The level of control by entomopathogenic nematodes decreased substantially with higher densities of weevils between the first and second year trials, suggesting that the ratio of nematodes to weevils may affect the level of control. *Steinernema feltiae* BU failed to provide greater than 24% control at concentrations of 2.5×10^9 IJs/ha, 70% lower than in the previous year. The concentration of nematodes did not have an effect on the density of *L. maculicollis* larvae in 2007 (2.5 and 1.25×10^9 IJs/ha) or 2008 (5, 2.5 and 1.25×10^9 IJs/ha), though a trend towards improved numerical control was observed. *Steinernema carpocapsae* BU produced a near significant control ($P = 0.062$) over the other treatments when the data were combined by species and concentration. In 2008,

both *S. carpocapsae* BU and *S. feltiae* BU provided significantly improved control when compared to *H. bacteriophora* BU and the untreated controls. Though statistical significance was not always found, the combination of these results and laboratory data (McGraw and Koppenhöfer 2008b) suggest that *S. carpocapsae* and *S. feltiae* have an effect on *L. maculicollis* larvae in the field.

This study suggests that nematodes released into short mown golf course fairways are unlikely to persist in consistent or high enough densities to significantly reduce future weevil generations. Although the general turfgrass environment may be amenable to the use of entomopathogenic nematodes in controlling soil dwelling insect pests, the physical properties of golf course turfgrass and the low aesthetic thresholds for damage may be less suitable for one time inoculative releases of entomopathogenic nematodes. The nematode populations in most treatments rapidly declined in the period between application and 14 d, increased between 14 and 28 DAT, before diminishing to almost undetectable levels by the time second generation weevil larvae were present in the soil. The initial decline in nematode killed bait insects is likely due to a combination of IJs entering hosts and succumbing to natural mortality factors. Fenton's (2000) review of published studies on the daily mortality rates of IJs revealed an average mortality rate of 0.16 per day, or that 90% of the released population would be lost after 14 d. The estimate includes various substrates (water, soil, sand) and settings (laboratory, field) and thus is more likely to underestimate the daily mortality of IJs in golf course soils. Nematode infection of bait insects increased between 14 and 28 DAT in many of the treatments in this study, suggesting that recycling occurred in the field despite a dramatic initial decline. However, the ability of nematodes to recycle in weevils between 14 and

28 DAT is of little practical value to turfgrass managers hoping to control of *L. maculicollis*, since by 28 DAT damage will have already occurred. It would be advantageous for released nematodes to persist in high enough densities to impact future generations (> 56 d). However, it is unlikely that entomopathogenic nematodes would do so, given that endemic populations dramatically crash between *L. maculicollis* first and second generations in New Jersey, due to low host encounters and extreme environmental conditions (McGraw and Koppenhöfer 2008a).

Timing, application strategy and concentration of nematodes are likely to have major consequences on the successful use of entomopathogenic nematodes to control *L. maculicollis* populations. Nematode applications were timed to coincide with the majority of the *L. maculicollis* larval populations entering the fourth instar, the first stage living entirely outside of the plant and susceptible to nematode infection. At this time many younger larvae are still present in the population, most likely protected in the plant or not in close contact with nematodes in the soil. The released nematode population density may be exhausted by the time the majority of the younger larvae develop to the fourth instar, especially if stages are staggered over several weeks, thus diminishing any effect of nematode treatment. Recently developed ecological models suggest that the strategy of application and characteristics of the system to which nematodes are applied are equally important as concentration (Fenton et al. 2000, 2001). Fenton et al. (2001) modeled pest suppression using entomopathogenic nematodes through three application strategies: inundative (one time), pre-emptive and repeated applications. Inundative control is predicted to best fit systems where pests have high reproductive capabilities, long-lived susceptible stages and the crop can sustain some injury while nematodes

recycle in hosts. Pre-emptive or "nematode in first" technique (Kaya 1993) works well if the application is accurately timed and the growth rate of the population is low. However, if nematode mortality is high, large concentrations are required, thereby making use of entomopathogenic nematodes more expensive. Repeated applications, similar to the split applications in this study, can be effective against pests with long-lived larval stages in crops with a high sustainable injury level. The daily nematode mortality and the interval between applications are critical to the success of repeated applications. The characteristics of the golf course turfgrass environment (low tolerance for sustaining injury, high daily nematode mortality) and the life history characteristics of *L. maculicollis* (short duration of larval stages, relatively low reproductive rate) do not exactly match the optimal criteria for any of the strategies.

An increase in *L. maculicollis* control was observed when applications were split into half concentrations delivered 1 wk apart in 2007. In 2008, the control was much lower than the previous year despite lower densities of *L. maculicollis* larvae. Fenton (2002) demonstrated high levels of control of sciarid flies in mushroom houses with smaller concentrations of nematodes in repeat applications compared to single applications. Modeling repeat applications suggests that optimal control with less inoculum is obtained when the second application is timed to coincide with the peak in pest densities. The variable control in the present study's repeat applications may have been due in part to the timing of the second application. A greater distribution of stages was detected in 2008 than in 2007 (data not shown). Therefore, a 1-wk interval between applications may have been good given the temporal distribution of the first generation larvae in 2007, but not in 2008. Future research should seek to determine the number of

applications needed to adequately control *L. maculicollis* based on the larval stage distribution.

The use of multiple-species releases in the biological control of insect pests has produced mixed results, with effects ranging from antagonism (Synder and Ives 2001) to synergy (Losey and Denno 1998). Positive outcomes are often found when combining species of generalist predators (Chang 1996; Sokol-Hessner and Schmitz 2002), including entomopathogenic nematodes (Neumann and Shields 2008). Biological control with entomopathogenic nematodes may be enhanced by multiple-species releases if nematodes attack a greater percentage of hosts by occupying different niches or separately attack the pest as it moves through different environments. *Listronotus maculicollis* larvae occupy different environments and exhibit different behaviors as they develop, initially dwelling inside the turfgrass plant, then occasionally moving between plants, and finally moving into the soil thatch matrix to feed externally upon the roots. Combining nematode species was originally intended to take advantage of the different foraging strategies of the two entomopathogenic nematodes tested (Grewal et al. 1994b). *Steinernema carpocapsae* employs an ambusher strategy, waiting to attack mobile prey. *Heterorhabditis bacteriophora* is a "cruiser" nematode that actively seeks a host and is capable of attacking more sedentary hosts. Combining these species resulted in greater control than either species alone. *Steinernema feltiae* employs an intermediate foraging strategy, employing both ambush and cruise behaviors to infect mobile and sedentary hosts. Its flexible foraging strategy may be the reason why *S. feltiae* has provided high levels of control in laboratory studies applied against *L. maculicollis* larvae in infested turf cores, as well as in the field.

The results found in this study indicate that the species of entomopathogenic nematodes currently available to turfgrass managers, at least those used in this study, and the endemic species isolated from *L. maculicollis* cadavers cannot provide consistent control at standard field rates. Entomopathogenic nematodes are not likely to be adopted by turfgrass managers, given the high demands for pristine playing conditions and the availability of cheaper, more reliable chemical options. However, should future research identify novel species and strains that provide higher, more reliable control, and may be mass produced, research will need to identify the factors contributing to the variable degree of control observed in past trials. Identifying the sources of variability from both the pest (spatial distribution, susceptibility) and applied nematodes (e.g., timing of application, concentration, number of applications) may contribute to the development of release strategies that lead to greater control with comparable or possibly lower concentrations.

Figure 4.1. Mean density (+/- SEM) of *Listronotus maculicollis* immature stages in golf course fairway small plots 14 d after treatment of the entomopathogenic nematodes nematodes *S. carpocapsae* (Sc), *H. bacteriophora* (Hb), *H. megidis* (Hm), *S. feltiae* (Sf), and *S. kraussei* (Sk) for field trials conducted in 2006, 2007, and 2008 (Experiment 1). Nematodes were commercial strains (BU = Becker Underwood) except for one field isolated (Hb PB). Figures above or within bars are percent reduction compared to untreated control (UTC). Means with same letters over bars in the 2008 trial (grouped as species) are not significantly different (Tukey's pairwise comparison, $P < 0.05$).

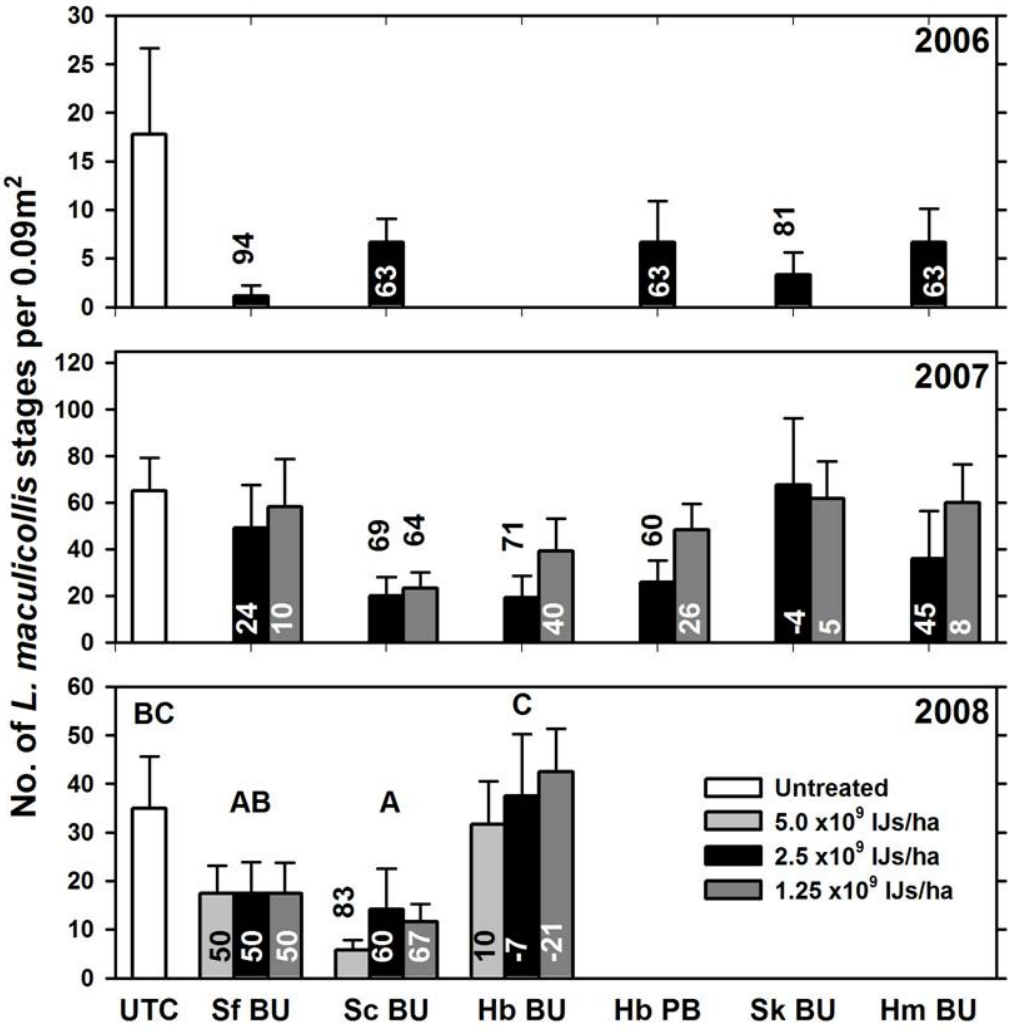


Figure 4.2. Field persistence of released nematodes in single treatments of 2.5×10^9 IJs/ha through 56 d after treatment estimated by the number of bait insects infected per treatment (Experiment 1). Asterisces (*) indicate significant mean separation (Tukey's pairwise comparison, $P < 0.05$).

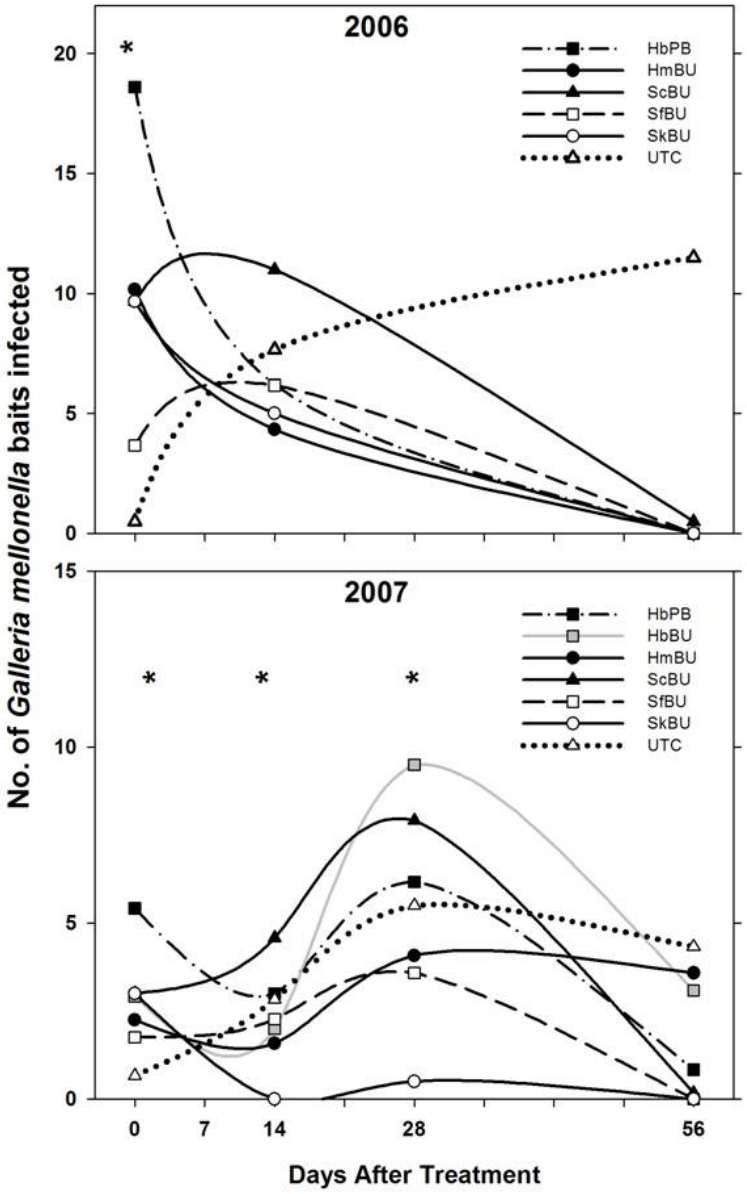


Figure 4.3. Mean density (+/- SEM) of *Listronotus maculicollis* immature stages in golf course fairway small plots 14 d after split application of the entomopathogenic nematodes *H. bacteriophora* (Hb PB) and *S. feltiae* (Sf BU) (Experiment 2). Treatments were a high (2.5×10^9 IJs/ha), low (1.25×10^9 IJs/ha) or split application (1.25×10^9 IJs/ha applied twice, 1 wk apart) (Experiment 2). Figures above or within bars are percent reduction compared to untreated control (UTC). Asterisces (*) indicate significant mean separation from the UTC (Tukey's pairwise comparison, $P < 0.05$).

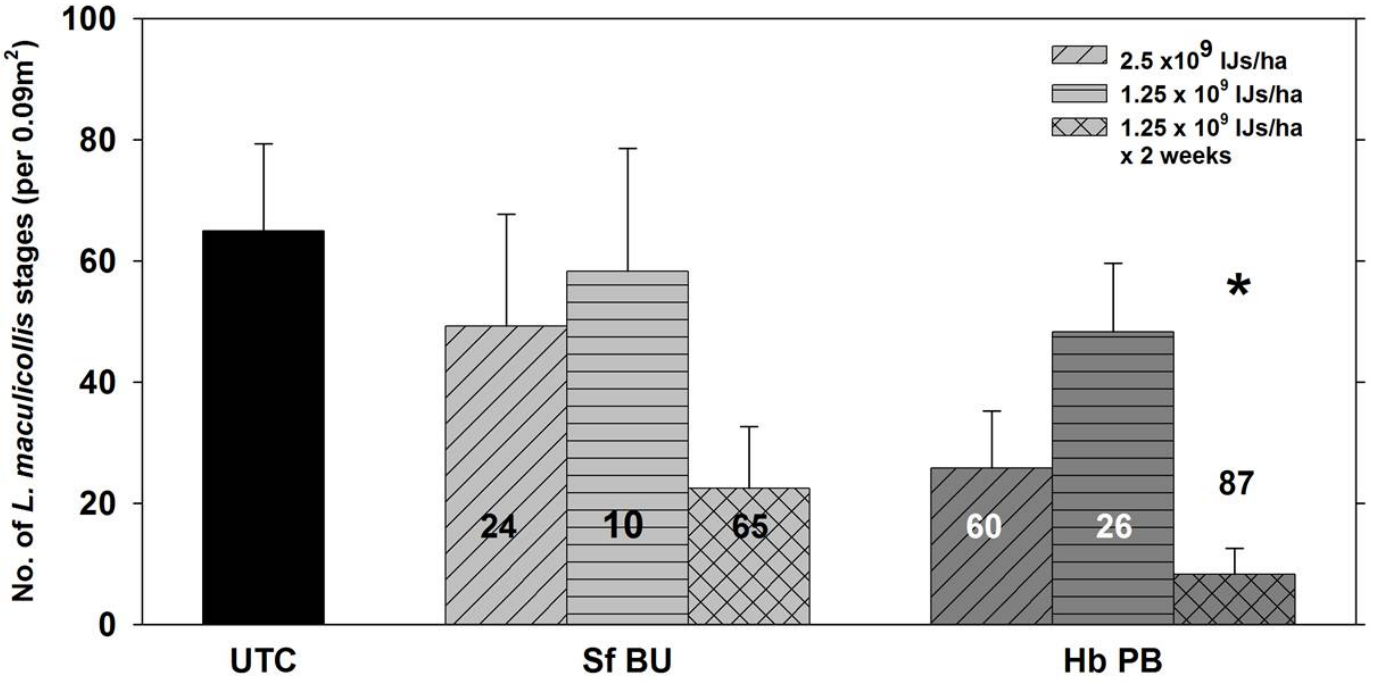


Figure 4.4. Number of bait insects infected by entomopathogenic nematodes in field releases of the treatments of *H. bacteriophora* (Hb PB) and *S. feltiae* (Sf BU) applied in single applications of 2.5×10^9 IJs'ha (H = high rate) or 1.25×10^9 IJs/ha (L = low rate), or a repeat application of 1.25×10^9 IJs/ha applied in two consecutive weeks (S = split application)(Experiment 2). Bars above columns represent standard error of the means.

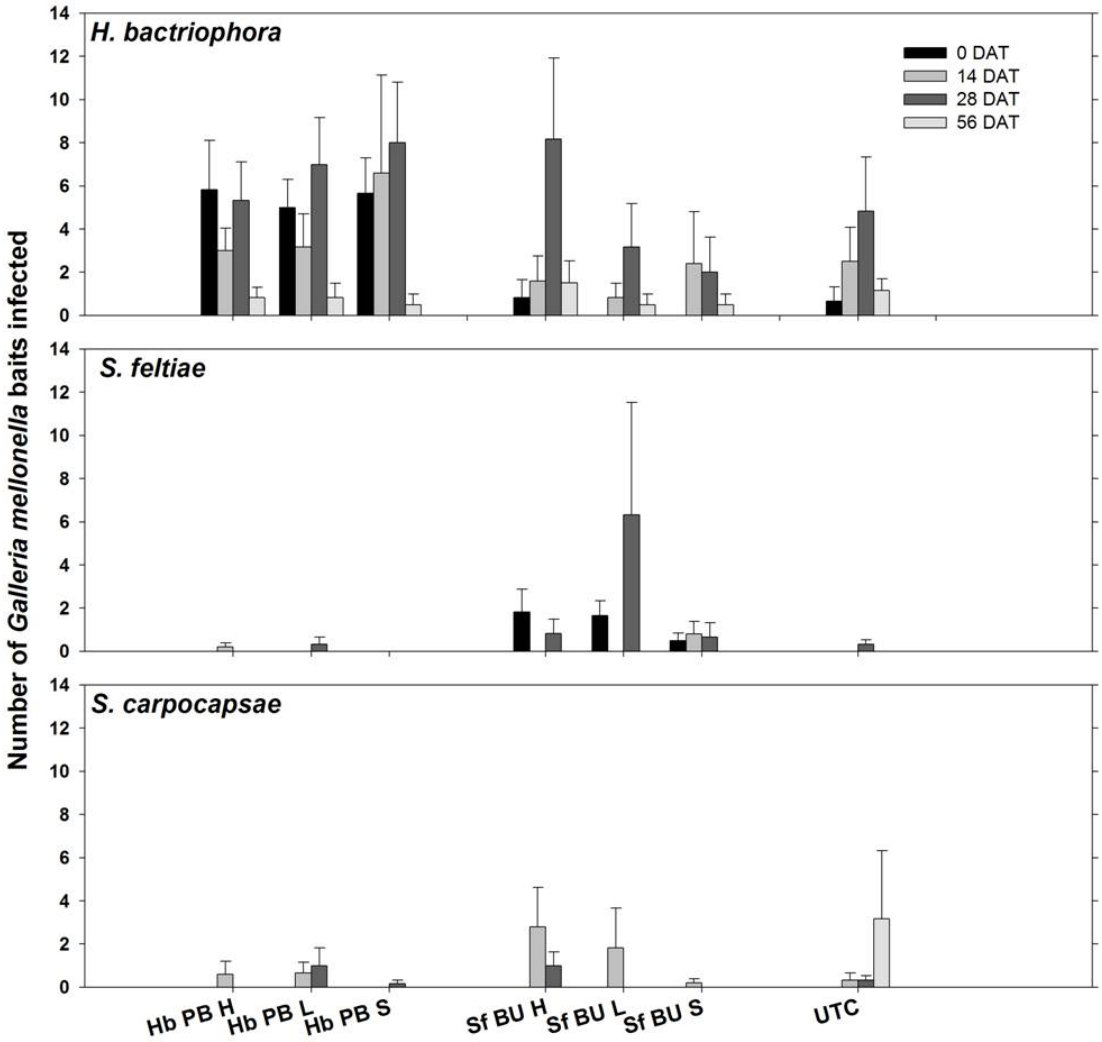


Figure 4.5. Mean density (+/- SEM) of *Listronotus maculicollis* immature stages in golf course fairway small plots 14 d after application of the entomopathogenic nematodes *H. bacteriophora* (Hb PB) and *S. carpocapsae* (Sc BU) (Experiment 3). Treatments were a high rate (2.5×10^9 IJs/ha), low rate (1.25×10^9 IJs/ha) or combination of both species at low rates (1.25×10^9 IJs/ha per species). Figures above or within bars are percent reduction compared to untreated control (UTC).

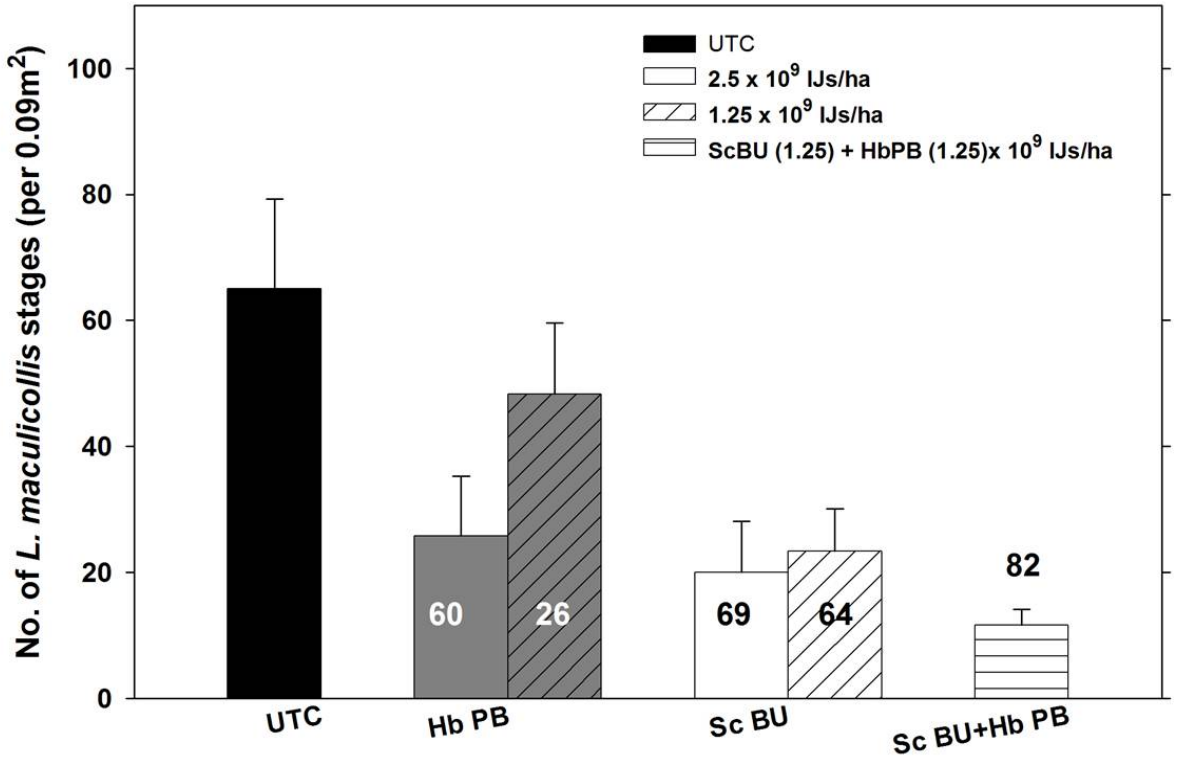
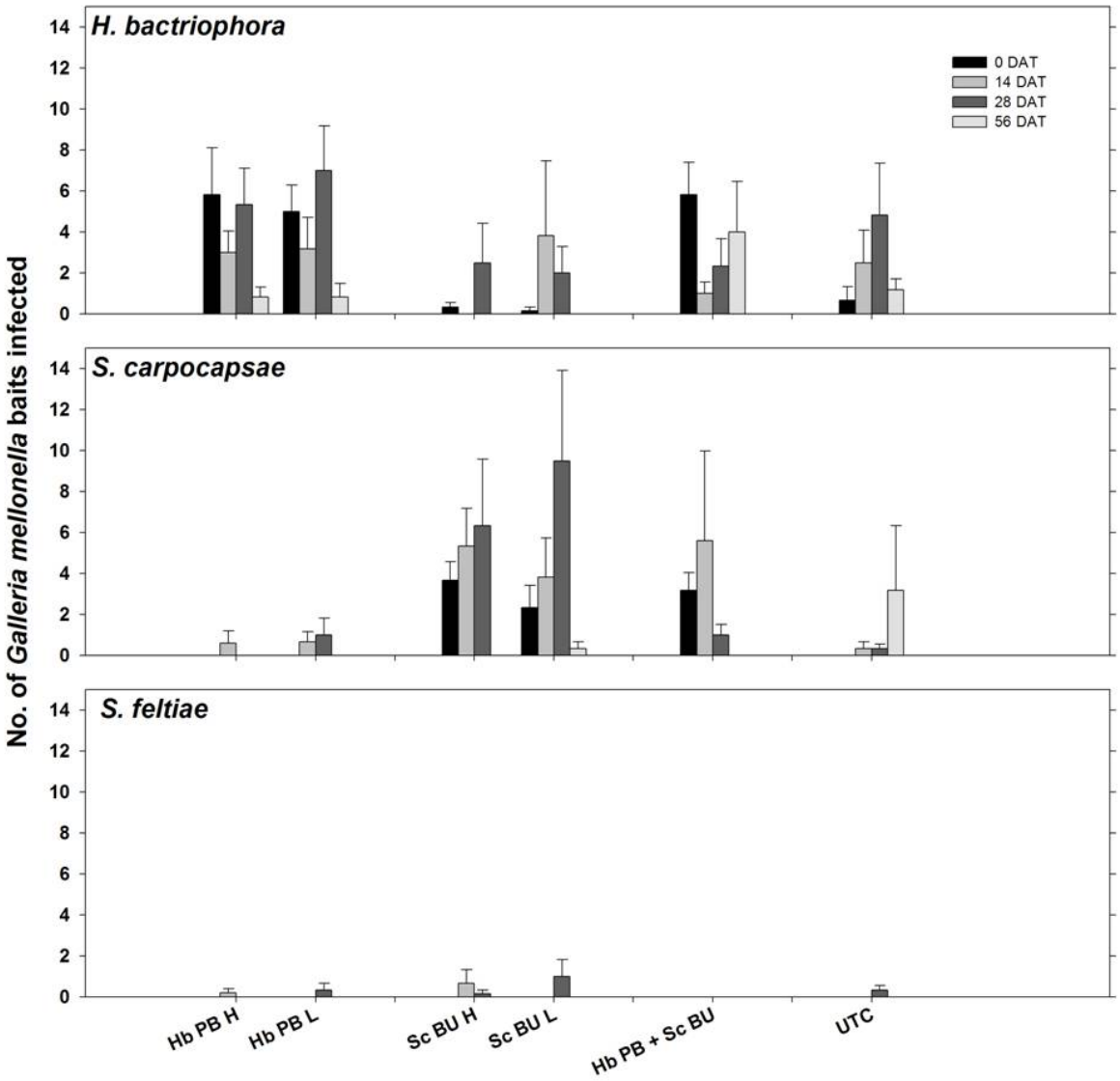


Figure 4.6. Number of bait insects infected by entomopathogenic nematodes in field releases of the treatments of *H. bacteriophora* (Hb PB) and *S. carpocapsae* (Sc BU) applied in single applications of 2.5×10^9 IJs/ha (H = high rate) or 1.25×10^9 IJs/ha (L = low rate), or combination of both species at low rates (1.25×10^9 IJs/ha per species)(Hb PB + Sc BU) (Experiment 3). Bars above columns represent standard error of the means.



CHAPTER FIVE

INTERPRETING DISPERSAL AND HOST SELECTION BY EMERGING *LISTRONOTUS MACULICOLLIS* ADULTS THROUGH SPATIAL ANALYSES: IMPLICATIONS FOR TARGETED PEST MANAGEMENT

Abstract

The long history of conducting preventive insect pest management in urban environments has caused for synthetic chemicals to be misused spatially and temporally. Additionally, this management style has generated gaps in our understanding of the behavioral ecology of insect pests within these environments. I characterized the spatio-temporal dynamics of overwintering adult *Listronotus maculicollis* populations colonizing golf course fairways with Spatial Analyses by Distance IndicEs (SADIE) to understand the behaviors governing their population dynamics and to better target management tactics. Spatial association analyses between *L. maculicollis* adults, their larvae and their preferred host plant (*Poa annua)* were conducted to understand dispersal, oviposition and host selection by adults. Adults randomly colonized and dynamically moved throughout fairways. However, cumulative captures were significantly aggregated along fairway edges closest to overwintering sites demonstrating progressive movement through the edges. The spatial patterns of cumulative captures of adults rather than weekly patterns were strongly associated with larvae, suggesting that the majority of eggs are deposited as adults arrive on the margins of fairways over the course of several weeks. Host plant species did not

have an effect on the distribution of either *L. maculicollis* stage which leads to questions regarding traditional assumptions of host preference for short mown *P. annua.* Instead, a conceptual model is proposed stating that the aggregated distribution of larvae is generated by a low encounter rate of short mown hosts, rather than preference for species or cultivar. This study indicates that caution need be applied when using preference-performance criteria in host preference studies. Future behavioral studies are needed to address the contributions that encounter rate and host species have on *L. maculicollis* host selection and oviposition. Addressing these factors could lead to the development of novel, sustainable management tactics for controlling *L. maculicollis* populations.

Introduction

There is a growing awareness to the unintended negative consequences that synthetic chemical pesticides may have on non-target insect communities, wildlife and the public. This awareness is especially heightened in urban environments where pest management is conspicuous and often without re-entry intervals. However, on golf courses the increased demand for fewer chemical inputs is often at odds with the public's demand for the highest aesthetic quality turfgrass. The low tolerance for pest damage has created a management culture revolving around preventive control strategies for insects and diseases and a deviation from the fundamental concepts of integrated pest management (IPM). The long history of preventive pest management on golf courses has led to large gaps in our understanding of basic pest behavior and ecology in these environments.

A better understanding of the behaviors that govern the structure and dynamics of pest populations can assist in the development of novel, more ecologically sensitive pest management strategies. However, direct observation of behavior is often difficult, especially with small and cryptic insects. Ecologists have taken several approaches to understand insect behaviors in field settings, from mark-recapture studies to mathematical modeling (Turchin 1991, 1996; Wiens et al. 1995). Many studies have inferred behavioral processes by studying the resulting spatial structure of populations (Taylor 1984). However, traditional analyses of the spatial structure of populations using mean to variance relationships have difficulty detecting complex spatial patterns and relationships between spatial patterns of multiple organisms (Perry 1995). Advances in statistical methodologies, particularly Spatial Distance by IndicEs (SADIE), have aided the detection of complex spatial patterns and association between multiple datasets (Perry 1998; Perry and Dixon 2002). SADIE has been used in a variety of ecological studies and has led to increased understanding of pest dispersal (Ferguson et al. 2000; Kim et al. 2007), predator-prey dynamics (Winder et al. 2001, 2005; Pearce and Zalucki 2006) and the influence of habitat management on insect abundance (Donovan et al. 2007). SADIE has also been used to develop sampling plans for the management of pests (Turechek and Madden 1999).

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is a severe pest of annual bluegrass (*Poa annua* L.), a grass species common in short mown areas on golf courses in the temperate Unites States (Vittum et al. 1999). If uncontrolled, populations of late instar larvae can damage turf in patches measuring several tens to hundreds of square meters. Therefore turfgrass managers attempt to prevent larval damage, relying

mainly on broad spectrum insecticides targeting adults prior to oviposition. The heavy reliance on preventive measures combined with a poor understanding of the spatio-temporal dynamics of *L. maculicollis* has contributed to the development of pyrethroid resistant populations in some areas within the weevil's distribution (Cowles et al. 2008).

Listronotus maculicollis has three generations per year in New Jersey, but the first generation typically is the most damaging and the focus of most control efforts (McGraw and Koppenhöfer 2007). Adults overwinter in protected habitats in the leaf litter surrounding trees or in tall grasses tens to hundreds of meters away from the shorter mown playing areas (Vittum 1980; Diaz and Peck 2007). Overwintering adult populations appear on short mown playing surfaces (e.g. tees, fairways, greens) in early April in the metropolitan New York City area. Mated females chew notches into the stem of short mown turfgrass plants, where they deposit their eggs either singly or in small batches. Larvae are stem borers in the first three instars, protected within the plant from most chemical insecticides. Fourth and fifth instars feed externally on the crown, severing the actively growing point of the turfgrass plant, and causing the most extensive damage to the turf.

Large gaps exist in our understanding of *L. maculicollis* behavior which limit the identification and development of novel control strategies. However, recent studies have enhanced our current understanding of *L. maculicollis* dispersal and host selection. Damage occurring from first generation larvae is typically located on the edges of short mown turfgrass stands bordering overwintering sites (e.g. tree lines, leaf litter, tall grasses). Turfgrass managers also observe that the edges of short mown areas that are primarily comprised of *P. annua* are selectively damaged, while neighboring grasses are

left undamaged. In a series of experiments Rothwell (2003) demonstrated that pattern of damage could be attributed to the dispersal of overwintering adults and the preference for short mown *P. annua*. Overwintering adults migrate from overwintering sites to short mown areas primarily by walking (Rothwell 2003), causing a progressive movement through the edges of short mown areas. Laboratory and field host preference studies have indicated that female *L. maculicollis* will oviposit more often into *P. annua* than other common golf course turfgrasses such as creeping bentgrass (*Agrostis stolonifera* L.) cultivars, perennial ryegrass (*Lolium perenne* L.) or Kentucky bluegrass (*Poa pratensis* L.) (Rothwell 2003). Additionally, larvae were shown to gain fitness advantages (reduced development time and increased weight) when developing on short mown *P. annua.* Furthermore, Rothwell's results indicate an oviposition bias towards shorter mown grasses of the same species or cultivar. Although these studies confirmed turfgrass manager's observations of *L. maculicollis* damaging edges of turfgrass with *P. annua* while adjacent grass species are left undamaged, recent reports of *L. maculicollis* damaging *A. stolonifera* on the edges of pure and mixed turfgrass stands (Rothwell and Vittum 2003) lead to questions about *L. maculicollis* host preference.

In this study, I characterized the spatio-temporal dynamics of unmanipulated, emerging *L. maculicollis* adult populations to better understand the behavioral processes governing dispersal and host preference. In particular, I sought to determine how the spatial and temporal patterns of emerging adults were associated with spatial patterns of larvae and hosts. This study should provide insight into the population dynamics of *L. maculicollis*, assist in the development of sampling plans to reduce the amount of pesticides applied in time and space, and stimulate future behavioral studies.

Materials and Methods

Study Sites and design

Listronotus maculicollis spatial and temporal distribution was monitored on two golf courses in northern New Jersey with histories of larval damage. Blocks were established on four separate fairways with mixed stands of *P. annua* and *A. stolonifera*. Two blocks ("11" and "12") were located at Brooklake Country Club (BLCC) (Florham Park, NJ) and two ("5W" and "6W") at Upper Montclair Country Club (UMCC) (Clifton, NJ). Each block measured 10.98 m ×14.64 m (length × width relative to the length of the fairway) and consisted of 48 1.83 × 1.83 m square sampling units. Each block had experienced damage to an edge of the fairway bordering potential weevil overwintering sites (long mown grasses and dense tree line/woods) in the previous year (referred to herein as "near edge"). The opposing edge of the fairway consisted of long mown grasses and individual trees (referred to herein as "far edge"). Blocks were arranged parallel to the rough/fairway border on the near edge of the block, extending across the entire width of fairway for Blocks 6W and 11 and less than 5 m from the far edge of rough/fairway border for Blocks 5W and 12 .

Sampling

The sampling period commenced 31 March 2008, a week prior to when traditional plant phenological indicators (*Forsythia* spp.) estimated that adults had begun emerging from overwintering sites (Tashiro et al. 1978). Adult sampling was conducted weekly using a reverse air flow vacuum/leaf blower (Homelite Vac Attack II, Anderson, SC), fit with a nylon mesh basket (324-mesh, 7.2 × 7.2 openings per cm^2) to capture

weevils before entering the engine (Rothwell 2003). Sampling was conducted between 0600 and 0900 h. Each sampling unit was vacuumed for 10 seconds, after which the basket was removed and its contents emptied onto a tray to count the number of *L. maculicollis* adults. Adults were returned to the sampling unit after counting. Weekly sampling ended when monitoring of larval populations in nearby plots showed that first instars were no longer being recruited into the population, indicating that the emerging adults had ceased ovipositing (11 May).

Larvae were sampled on 19 May by removing six soil cores (5.4 cm diameter × 5 cm depth) from each sampling unit using a soil corer (Turf Tec International, Oakland Park, FL). Soil samples were placed in a polyethylene bag, sealed, and transported to the laboratory in a cooler. In the laboratory, larvae were extracted from soil cores through heat (29 to 32°C for 7 d) followed by manual examination of the plant material, thatch and soil, allowing for a more efficient sampling of young and old larvae (Diaz 2006; B. McGraw unpublished data).

The percentage of *P. annua* within each sampling unit was estimated on 13 April using a 30.5 × 30.5 cm sampling frame divided into 25 sub-squares with monofilament line. The frame was randomly tossed within each sampling unit and the dominant turfgrass species within each sub-square was identified and recorded.

Spatial analysis

The spatial distributions of *P. annua*, *L. maculicollis* adults and larvae were characterized using Spatial Distance by IndicEs (SADIE) (version 1.5, K.F. Conrad and IACR Rothamsted Experimental Station, Harpenden, Herts, U.K.). SADIE characterizes the degree of clustering of counts by an index of aggregation (I_a) calculated through

randomized permutations of the counts (5967 for this study) (Perry, 1995; Perry, 1998). Clusters can be in the form of patches (large counts in close proximity) or gaps (zeroes or small counts in close proximity). I_a values equal to, greater than, and less than 1 indicate a random, aggregated, and uniform distribution, respectively. The randomization procedure allows for significance testing on the calculated index's departure from randomness (P_a). The null hypothesis may be rejected when $P_a < 0.025$ (accept the alternative hypothesis for aggregation) or $P_a > 0.975$ (accept the alternative hypothesis for uniformity). SADIE calculates each sample's contribution to local clustering through the unitless sub-indices v_i and v_j. v_i values greater than 1 contribute to patches and v_j values less than 1 contribute to gaps. v_i and v_j values were used to develop contour maps of the spatial distribution of adults in consecutive weeks, cumulative adult captures, larvae and *P. annua* using SigmaPlot (version 8.0).

Additionally, SADIE allows testing for spatial associations between two sets of data (Perry and Dixon, 2002). SADIE calculates an index of association (X) based on similarities between local spatial values at corresponding sample points between datasets and generates an associated probability of the index's departure from randomness (P_x). X values are greater than zero for associated populations, less than zero for dissociated populations, and around zero for populations that are randomly distributed to one another. The index was used to test for spatial similarities between *L. maculicollis* larvae and adults and between both weevil stages and *P. annua*. The spatial association between adult distributions in successive sampling bouts within the same block were compared to assess the relative stability of the spatial pattern and to infer movement within the block. Additionally, Pearson's correlations were performed to compare the relationships

between adult counts and larval densities and the relationship between adult aggregation values and density.

Results

Spatial distribution

Poa annua was significantly aggregated on fairways edges in two of the four blocks ($I_a \geq 1.561$, $P_a \leq 0.007$) (Fig. 5.1). However, the percentage of *P. annua* in three of the four blocks exhibited sharp gradient from high densities near edges to low in the centers of fairways (Table 5.1). *Poa annua* in blocks 5W and 6W were randomly distributed ($I_a \leq 1.024$; $P_a \geq 0.366$) despite the appearance of uniformity in 5W (95% block average). *Poa annua* density in Block 6 was relatively low (16% block average) though higher densities were found along the near edge (54%).

Adults colonized fairways between 6 April and 20 April (Fig. 5.2), peaking in abundance in all blocks on 5 May with a smaller peak observed on 20 April (Fig. 5.3). Adult spatial pattern was not significantly different from a random distribution upon colonization of fairways with the exception of Block 5W. Twenty contour plots were developed for weekly adult aggregation, of which only two were statistically aggregated (Block 5W; 20 April and 5 May; $I_a > 1.563$; $P_a < 0.007$). Adults were found in relatively low numbers, ranging from 0 to 7 per sample unit (0 to 2.09 adults per m^2) which could have led to an inability to detect significant aggregations. Despite the low capture numbers, I_a values were observed to significantly increase with the increase in density of adults ($r = 0.664$, $P = 0.001$).

Cumulative captures of adults were significantly aggregated along the edges of three of the four fairways ($I_a > 1.455$; $P_a < 0.019$), though not always on the edges

nearest to suspected overwintering sites (Fig. 5.1). Cumulative adult captures in two blocks (Blocks 6W and 12) were aggregated along both the near and the far edges. Visual examination of the temporal dynamics of adult aggregation combined with the cumulative captures by row within Blocks 6W and 12 (Table 5.1) indicate that adults immigrated into these fairways from two separate overwintering populations. Analysis of the cumulative captures of adults by row in the remaining two blocks showed greatest captures occurring closest to the near edge, diminishing with distance away from edges, thus demonstrating a progressive movement through the near edge by adults from one overwintering site.

Listronotus maculicollis larvae were significantly aggregated in three of the four blocks (Fig 5.1). Aggregations occurred on near edges on all three blocks as well as the far edge on Block 12. Larval spatial distribution was not significantly different from a random distribution in Block 6W, where high densities of larvae were found on near and far edges of the block, lessening in the center of the block. A gradient of larval density away from near edges were observed in blocks where one overwintering population colonized the block (Blocks 5W and 11) and converging gradients away from the edges in populations with two emerging overwintering populations (Blocks 6W and 12).

Association analyses

Spatial association analyses between the spatial patterns of adults in consecutive weeks suggest that adult movement within fairways is dynamic. Only one instance of stability in the spatial pattern of adults in consecutive weeks was found (Block 11; X = 0.329, P = 0.018), occurring in the final two weeks of the study (Table 5.2). Adult spatial

stability in Block 12 neared significance in the first two weeks of the monitoring program ($X = 0.615$, $P = 0.027$).

Spatial analyses did not indicate that adults aggregate in areas where *P. annua* is aggregated despite most blocks exhibiting an edge effect of *P. annua* density and cumulative adult captures occurring on the edges of fairways. Significantly associated as well as dissociated relationships were found between the weekly distributions of adults and *P. annua.* All of the significant associations occurred in Block 11 where *P. annua* was aggregated along the near edge of the block (Table 5.3). Cumulative adult captures were also only significantly associated with *P. annua* in Block 11.

Significant spatial associations were detected between adults and larvae early in the observation period in blocks where *P. annua* was aggregated (Blocks 11 and 12) ($X \geq 0.366$, $P \leq 0.023$), as well as in the later weeks in blocks where *P. annua* was aggregated or randomly dispersed (Blocks 5W and 12) (Table 5.4). The spatial distribution of cumulative captures of adults across the period was significantly associated with larvae in three of four blocks ($X \geq 0.356$, $P \leq 0.006$) but was not significant in the fourth block ($X \geq 0.221$; $P = 0.067$), suggesting that most eggs are deposited over several weeks rather than in a discrete time period. However, adult distribution during the peak in abundance (4 May) was significantly associated with larvae in one block ($X = 0.566$; $P < 0.0001$), indicating that a majority of eggs may have been deposited at this time in this block.

The relationship between cumulative counts of adults and larvae was further confirmed by correlation analyses. The cumulative counts of adults per row (see Table 5.1) and the resulting larvae were not correlated when the data from all blocks were combined ($r = 0.351$; $P = 0.092$). However, separating the data by the number of

overwintering populations entering the blocks resulted in a strong relationship between adult captures and larval densities when one population colonized the block (r = 0.765, *P* = 0.004) but no relationship when two overwintering adult populations converged (r = 0.428; *P* = 0.165).

Positive spatial association between *P. annua* and *L. maculicollis* larvae was found in blocks where both were aggregated, though only statistically significant in Block 11 ($X = 0.503$; $P < 0.001$) (Table 5.4). Significant spatial associations were not found when preferred hosts were random to uniformly distributed. Larvae in Block 5W, where *P. annua* was nearly uniform, were significantly aggregated along the near edge of the block. These results suggest that the aggregated oviposition on fairway edges might be due to reasons other than the distribution of hosts.

Discussion

This study has provided much greater insight into the dynamics of fairway colonization by overwintering adults than previous studies on the population dynamics of *L. maculicollis* (Rothwell 2003; Diaz 2006; McGraw and Koppenhofer 2008a). Little is known about the behaviors governing the population dynamics of *L. maculicollis,* especially those that relate to dispersal, foraging and oviposition. The scarcity in these kinds of data thus far has been due to an inability to rear the insect in the laboratory and difficulty of observing the weevil in field studies (Rothwell 2003). SADIE analyses have made it possible to infer some of these behaviors by characterizing spatial and temporal structure of adult populations and to test the strength of associations to larvae and host plants. However, even with significance testing, caution needs to be applied when

inferring causation since many complex interactions affect the spatial distribution of insects (Jones 1977). With this in mind, a conceptual model to colonization and oviposition within fairways by *L. maculicollis* can be developed and highlight areas where future research is needed.

Adult spatial distribution was shown to be unstable indicating that adults actively move into and randomly forage within fairways, rather than conforming to the spatial pattern of preferred host plants. The numbers of adults captured per sample were relatively low, which may have caused difficulties in detecting significant aggregations if traditional indices of aggregation based on mean to variance relationships had been used. Contour analysis of non-significant aggregations revealed that adults entered through the fairway margins, and in some instances through opposite edges. Also, the abundance and aggregation of adults along edges occurred on two separate dates, indicating an extended period of emergence from overwintering sites. The movement through the edge of fairways was first suggested by Vittum (1980) who hypothesized that damage was occurring along edges through progressive movement through and oviposition on fairway margins. The datasets further support this hypothesis, as cumulative captures of adults and larvae showed strong significant aggregations on fairway edges.

Earlier efforts to characterize adult spatial structure and movement may have been affected by number and location of overwintering sites in relation to the sampling area. Recent research investigating overwintering site selection by *L. maculicollis* has changed our traditional beliefs that the weevil prefers to overwinter in tree leaf litter (Diaz and Peck 2007). This belief was largely supported by observations of damage occurring along the edges of short mown playing surfaces adjacent to woods or tree lines. In two of

the four blocks, converging populations of overwintered adults emerged on to the same fairway leading to a more uniform distribution of adults and larvae in one block and two aggregations of adults on others. Had spatial mapping of the populations not been used, it would have been difficult to detect emergence from converging populations. Additionally, these data also demonstrate that adults produced in later generations do not orient towards one dominant tree line or cardinal direction when returning to overwintering sites.

I characterized the spatial distribution of larvae to gain insight into ovipositional choices by *L. maculicollis*, identify the time period when a majority of eggs are deposited and determine the effect of the spatial distribution of preferred hosts on the distribution of eggs. The spatial pattern of cumulative counts of adults showed the strongest significant association with the resulting pattern of larvae, suggesting that, similar to adult emergence, oviposition is likely to occur over several weeks. The progressive movement of adults and the deposition of eggs along the edges of fairways may indicate that females are mated before arriving on the shorter mown playing surfaces. Previous research on the reproductive seasonality of *L. maculicollis* has shown that between 5 and 55% of females can be inseminated prior to overwintering (Rothwell 2003). Future studies are needed to determine how mates are located and when and where mating occurs. Understanding these behaviors could lead to the development of new strategies that aim to disrupt mating and reduce egg laying on fairways.

The strong edge bias observed in the distribution of larvae appeared to be independent of the spatial distribution of *P. annua*. It could be expected that adult and larval distribution would be affected by the spatial pattern of *P. annua* since laboratory

results have indicated larval fitness advantages to developing on this species (Rothwell 2003). Optimal oviposition theory states that phytophagous insects that produce immobile offspring should maximize their fitness and discriminate between hosts that provide maximum benefits to their offspring (i.e., preference-performance theory) (Jaenike 1978). However, there is mounting evidence that this theory does not hold true for many insects (Courtney and Kibota 1990; Valladares and Lawton 1991) especially in instances where optimal foraging strategies are at odds with optimal oviposition choices (Scheirs and De Bruyn 2002). *Listronotus maculicollis* foraging strategies leading to aggregated oviposition along fairway edges could be caused by several factors independent of larval performance, including egg load or oviposition occurring at feeding sites (Prokopy et al. 1994; Scheirs et al. 2000).

The current understanding of *L. maculicollis* host preference is based on manipulated studies using preference-performance criteria for turfgrass species and mowing height (Rothwell 2003). Rothwell's studies showed a preference for *P. annua* over other common turfgrass species and a bias for shorter mown grasses compared to longer within the same species. It remains unknown as to whether host species or mowing height is a greater criterion for *L. maculicollis* host selection. These studies were conducted in the laboratory or small field plots where efforts to locate hosts can be assumed to be minimal. To fully understand host preference it is critical to determine the insect's encounter rate with hosts (Thomas and Singer 1987). *Listronotus maculicollis* adults may walk tens to hundreds of meters to short mown turfgrasses from overwintering sites (Diaz 2006). If mowing height is more important than cultivar in host selection, females would potentially encounter several thousand less acceptable hosts

before encountering a host that would provide the greatest benefits to their offspring. The low encounter rate may cause more eggs to be deposited on the edges of fairway margins independent of turfgrass species or cultivar. Complicating this matter is that *P. annua* is likely to be aggregated on the edges of heterogeneous stands with bentgrasses for its ability to tolerate increased levels of compaction and abrasion from mowing equipment traveling through and turning on fairway edges (D. Oatis personal communication). Furthermore, damage is expressed at lower larval densities in *P. annua* than in bentgrass (B. McGraw unpublished data), which could cause the appearance of preference when neighboring bentgrasses are unaffected, despite similar densities of larvae. Therefore, conclusions of host preference for *P. annua* based on observation of damage and biased edge densities may be inaccurate if females choose short mown hosts rather than *P. annua*.

This study demonstrates that though *L. maculicollis* adults dynamically disperse into and within fairways, first generation larvae are strongly aggregated along the edges. Currently, many turfgrass managers apply preventive chemical insecticides to all short mown areas targeting emerging overwintering adults. The data presented in this study suggest that adults emerge and oviposit over several weeks, thus requiring multiple applications or long residual action of controls to large areas of the course. Spatial mapping demonstrated that many areas within the centers of fairways contain low densities or no larvae, and thus do not require treatment. If this scenario bears out on most golf courses then curative controls could be targeted along the perimeters, thereby reducing the cost and risk of poorly timed preventive controls. However, sampling plans will need to be developed if curative controls are to be effectively targeted.

Characterizing the spatial distribution of pests is essential first step in the development of sampling plans. Given that adults are likely to be found in low densities, efforts to develop monitoring programs to either time preventive controls or estimate the need for curative action should take advantage of the higher adult captures and strong correlation between adults and larvae along edges of fairways. Finally, future research should strive to understand *L. maculicollis* behavior to develop novel management strategies.

Table 5.1. The number of *Listronotus maculicollis* adults captured between 31 March and 11 May 2008, density of *L. maculicollis* larvae (per 0.1 m^2) and *Poa annua* composition summarized by distance from the edge of the fairway border closest to the known overwintering site.

Block	**Distance from Near Edge**	**Adults (n)**	**Larvae (0.1 m^2)**	***Poa annua***
5W	0 to 1.8 m	35	172.5	96.9%
	1.8 to 3.6 m	27	120.0	96.9%
	3.6 to 5.4 m	18	71.7	95.5%
	5.4 to 7.2 m	17	80.0	95.3%
	7.2 to 9.0 m	11	48.3	90.6%
	9.0 to 10.8 m	2	36.7	97.7%
6W	0 to 1.8 m	29	409.2	54.7%
	1.8 to 3.6 m	13	246.9	3.1%
	3.6 to 5.4 m	18	260.7	9.8%
	5.4 to 7.2 m	4	270.0	13.3%
	7.2 to 9.0 m	20	284.2	7.0%
	9.0 to 10.8 m	17	344.0	11.7%
11	0 to 1.8 m	30	46.7	81.3%
	1.8 to 3.6 m	17	17.1	32.0%
	3.6 to 5.4 m	7	14.2	15.2%
	5.4 to 7.2 m	6	22.5	5.5%
	7.2 to 9.0 m	13	14.3	3.9%
	9.0 to 10.8 m	9	9.0	1.6%
12	0 to 1.8 m	15	30.0	45.3%
	1.8 to 3.6 m	9	26.7	14.1%
	3.6 to 5.4 m	18	11.7	16.1%
	5.4 to 7.2 m	9	16.5	18.0%
	7.2 to 9.0 m	12	24.2	1.6%
	9.0 to 10.8 m	18	27.8	7.0%

Table 5.2. Temporal stability of adult *Listronotus maculicollis* captures. *X* is the measure of the overall spatial association between the adult spatial patterns in consecutive weeks. *P* is a test of the spatial association's departure from randomness. *X* values greater than 1 are considered significantly associated when $P < 0.025$ (highlighted in bold).

	5W		**6W**		**11**		**12**	
Sampling dates	***X***	***P***	***X***	***P***	***X***	***P***	***X***	***P***
6 April to 13 April	n/a	n/a	n/a	n/a	n/a	n/a	0.615	0.027
13 April to 20 April	n/a	n/a	-0.057	0.607	0.328	0.057	-0.168	0.817
20 April to 27 April	0.120	0.235	-0.051	0.597	-0.261	0.953	-0.210	0.921
27 April to 4 May	-0.088	0.674	0.121	0.225	-0.029	0.563	-0.113	0.770
4 May to 11 May	0.177	0.118	-0.182	0.887	**0.329**	**0.018**	0.132	0.180

Table 5.3. Spatial association analyses between weekly and cumulative adult *Listronotus maculicollis* captures and *Poa annua.* *X* is the measure of the overall spatial association between counts and *P* is a test of the spatial association's departure from randomness. *X* values greater than 1 are considered significantly associated when $P < 0.025$ and significantly dissociated when $P > 0.975$ (highlighted in bold).

	5W		**6W**		**11**		**12**	
Sampling date	***X***	***P***	***X***	***P***	***X***	***P***	***X***	***P***
31 March	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6 April	n/a	n/a	n/a	n/a	n/a	n/a	0.304	0.188
13 April	n/a	n/a	0.032	0.417	**0.510**	**< 0.0001**	-0.103	0.690
20 April	-0.154	0.831	0.235	0.057	**0.580**	**< 0.0001**	-0.134	0.723
27 April	0.060	0.360	0.022	0.444	0.118	0.205	**-0.560**	**1.000**
4 May	0.139	0.193	0.149	0.165	0.280	0.027	0.155	0.198
11 May	0.134	0.188	0.133	0.183	0.232	0.053	-0.069	0.637
Cumulative	0.015	0.548	0.245	0.050	**0.356**	**0.006**	-0.154	0.804

Table 5.4. Spatial association analyses between counts of *Listronotus maculicollis* larvae and weekly or cumulative counts of *L. maculicollis* adults or density of *Poa annua*. *X* is the measure of the overall spatial association between counts and *P* is a test of the spatial association's departure from randomness. *X* values greater than 0 are considered significantly associated when $P < 0.025$ (highlighted in bold).

		5W		6W		11		12	
Observation	**Sampling dates**	***X***	***P***	***X***	***P***	***X***	***P***	***X***	***P***
Adults	**31 May**	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	6 April	n/a	n/a	n/a	n/a	n/a	n/a	0.334	0.088
	13 April	n/a	n/a	0.201	0.083	**0.338**	**0.023**	**0.655**	**0.003**
	20 April	0.235	0.082	0.077	0.294	**0.364**	**0.015**	0.205	0.101
	27 April	-0.192	0.885	0.158	0.148	0.197	0.100	-0.003	0.510
	4 May	**0.566**	**<0.001**	0.139	0.180	0.206	0.085	0.240	0.056
	11 May	0.104	0.260	0.207	0.091	0.154	0.173	**0.390**	**0.019**
	Cumulative	**0.540**	**<0.001**	**0.356**	**0.006**	0.221	0.067	**0.452**	**0.001**
***P. annua* density**	**13 April**	0.061	0.359	0.153	0.157	**0.503**	**<0.001**	0.277	0.045

Figure 5.1. Spatial distribution of cumulative counts of *Listronotus maculicollis* adults, larvae, and *Poa annua* within four sampling blocks. Maps were developed by contouring SADIE local clustering values from each of the 48 sample points per block. A local cluster value of 1.5 (red) indicates significant clustering of large counts close to one another, whereas a value of -1.5 contributes significantly to a gap (zeroes or low counts close to one another). I_a and P_a values beneath the map indicate the aggregation value of the overall spatial pattern and the associated significance test of the spatial pattern's departure from randomness. I_a values greater to, less than, and equal to 1 indicate a aggregated, uniform and random distribution, respectively. Significant spatial aggregation and uniformity is assumed at the $P < 0.025$ and > 0.975 level, respectively.

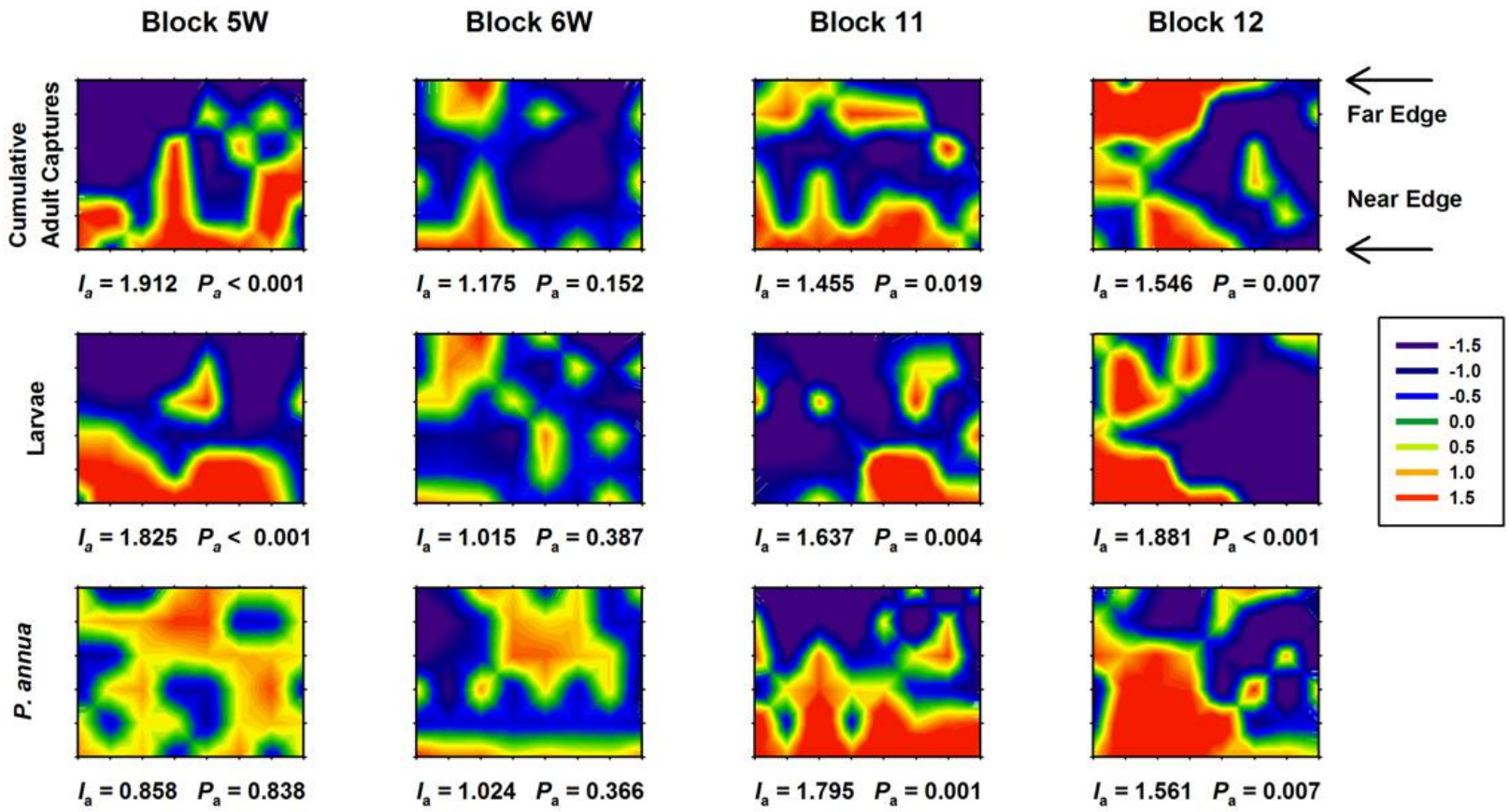

Block 5W
Block 6W
Block 11
Block 12
Cumulative Adult Captures
Larvae
P. annua
Far Edge
Near Edge
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
I_a = 1.912 P_a < 0.001
I_a = 1.175 P_a = 0.152
I_a = 1.455 P_a = 0.019
I_a = 1.546 P_a = 0.007
I_a = 1.825 P_a < 0.001
I_a = 1.015 P_a = 0.387
I_a = 1.637 P_a = 0.004
I_a = 1.881 P_a < 0.001
I_a = 0.858 P_a = 0.838
I_a = 1.024 P_a = 0.366
I_a = 1.795 P_a = 0.001
I_a = 1.561 P_a = 0.007

Figure 5.2. Time series of the spatial distribution of *Listronotus maculicollis* adults in weekly sampling bouts. Maps were developed by contouring SADIE local clustering values from each of the 48 sample points per block. A local cluster value of 1.5 (red) indicates significant clustering of large counts close to one another, whereas a value of -1.5 contributes significantly to a gap (zeroes or low counts close to one another). I_a and P_a values beneath the map indicate the aggregation value of the overall spatial pattern and the associated significance test of the spatial pattern's departure from randomness. I_a values greater to, less than, and equal to 1 indicate a aggregated, uniform and random distribution, respectively. Significant spatial aggregation and uniformity is assumed at the $P < 0.025$ and > 0.975 level, respectively.

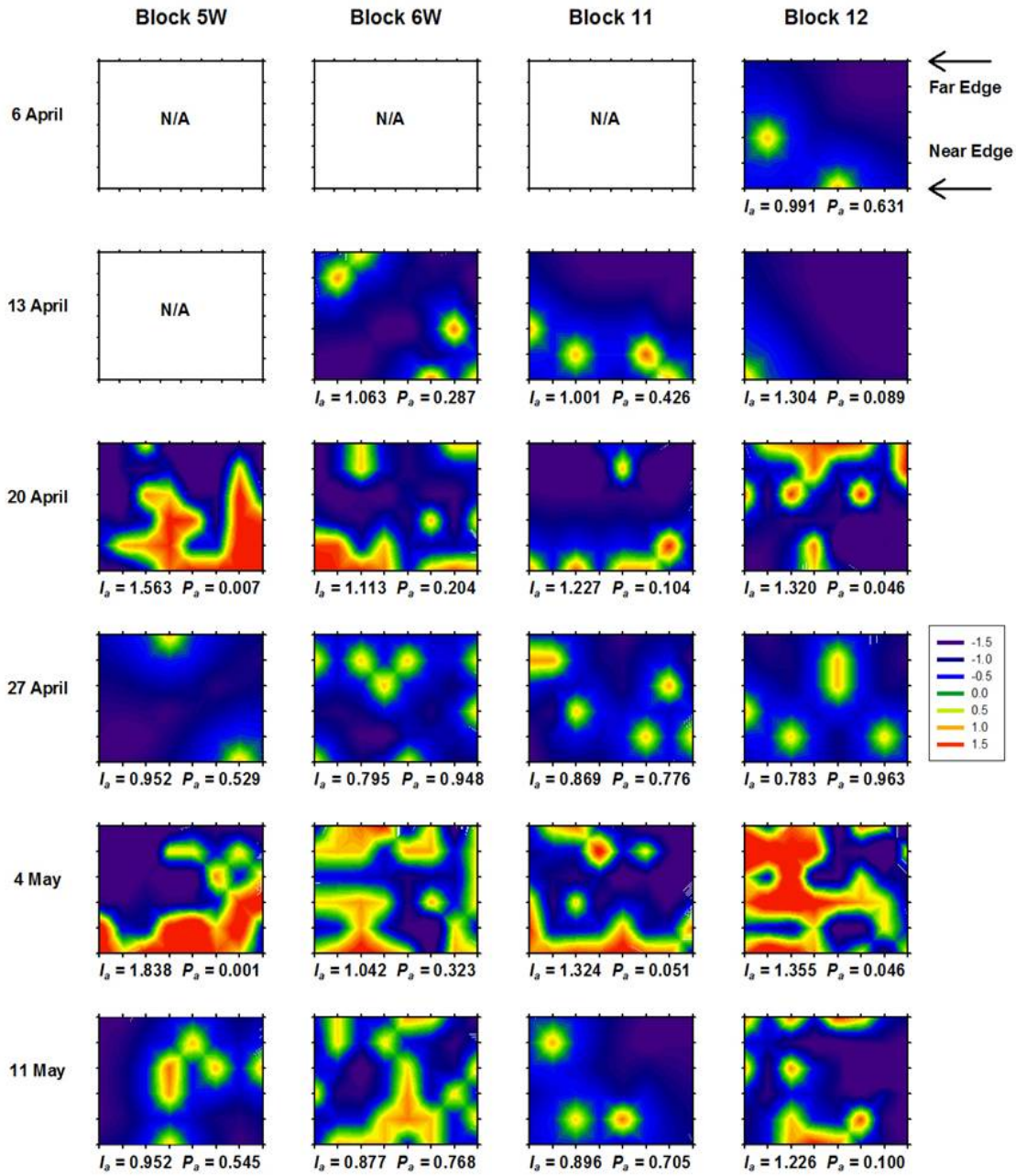
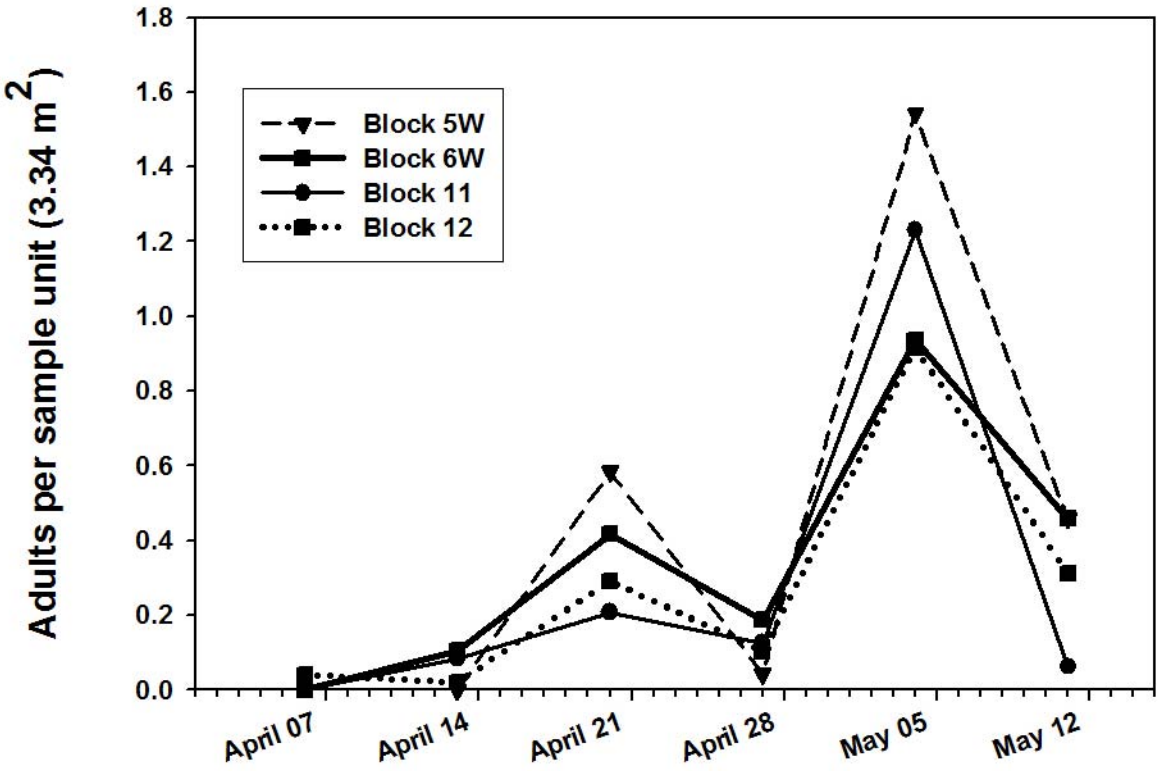

Block 5W
Block 6W
Block 11
Block 12
6 April
13 April
20 April
27 April
4 May
11 May
N/A
Far Edge
Near Edge
I_a = 0.991 P_a = 0.631
I_a = 1.063 P_a = 0.287
I_a = 1.001 P_a = 0.426
I_a = 1.304 P_a = 0.089
I_a = 1.563 P_a = 0.007
I_a = 1.113 P_a = 0.204
I_a = 1.227 P_a = 0.104
I_a = 1.320 P_a = 0.046
I_a = 0.952 P_a = 0.529
I_a = 0.795 P_a = 0.948
I_a = 0.869 P_a = 0.776
I_a = 0.783 P_a = 0.963
I_a = 1.838 P_a = 0.001
I_a = 1.042 P_a = 0.323
I_a = 1.324 P_a = 0.051
I_a = 1.355 P_a = 0.046
I_a = 0.952 P_a = 0.545
I_a = 0.877 P_a = 0.768
I_a = 0.896 P_a = 0.705
I_a = 1.226 P_a = 0.100
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

Figure 5.3. Average number of adult *Listronotus maculicollis* per sampling unit (3.34 m^2) in vacuum sampling between 31 March and 11 May, 2008.



CHAPTER SIX

BINOMIAL SEQUENTIAL SAMPLING PLANS FOR FORECASTING *LISTRONOTUS MACULICOLLIS* (COLEOPTERA: CURCULIONIDAE) LARVAL DAMAGE TO GOLF COURSE TURFGRASS

Abstract

Binomial sequential sampling plans were developed to forecast annual bluegrass weevil, *Listronotus maculicollis* Kirby, larval damage to golf course turfgrass and aid in the development of Integrated Pest Management (IPM) programs for the weevil. Populations of emerging overwintered adults were sampled over a 2-year period to determine the relationship between adult counts, larval density, and turfgrass damage. Larval density and composition of preferred host plants (*Poa annua* L.) significantly affected the expression of turfgrass damage. Multiple regression indicates that damage may occur in moderately mixed *P. annua* stands with as few as 10 larvae/0.09m^2. However, more than 150 larvae were required before damage became apparent in pure *Agrostis stolonifera* L. plots. Adult counts during peaks in emergence as well as cumulative counts across the emergence period were significantly correlated to future densities of larvae. Eight binomial sequential sampling plans based on two tally thresholds for classifying infestation (T = 1 and 2 adults) and four adult density thresholds (0.5, 0.85, 1.15 and 1.35 per 3.34 m^2) were developed to forecast the likelihood of turfgrass damage using adult counts during peak emergence. Resampling for Validation of Sample Plans (RVSP) software was used to validate sampling plans

with field-collected datasets. All sampling plans were found to deliver accurate classifications (correct decisions were made between 84.4 and 96.8 %) in a practical timeframe (average sampling cost less than 22.7 minutes). Four sampling plans, based on optimal tally threshold and critical proportion combination for each action threshold are presented and their levels of risk in forecasting *L. maculicollis* larval densities are discussed.

Introduction

Sampling is regarded as the cornerstone of Integrated Pest Management (IPM) and is essential to understanding the risks associated with increasing pest densities (Buntin 1994). However, it is an often overlooked step for managing insect pests in systems where aesthetic damage thresholds are low and inexpensive synthetic insecticides are prevalent. The long history of preventive insect pest management on golf courses has created a culture that is dependent on synthetic pesticides and in some extreme cases caused the development of insecticide resistant populations (Cherry and Nagata 2007; Cowles et al. 2008). The increased public demand for the adoption of IPM based programs and decreased use of synthetic pesticides in urban environments requires that researchers develop monitoring strategies that allow pest managers to accurately and efficiently assess pest populations.

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is a highly destructive pest of short mown turfgrasses on golf courses, tennis and lawn bowling courts in the mid-Atlantic and northeastern United States and eastern Canada (Vittum et al. 1999; Simard et al. 2007). Populations in the epicenter of the weevil's distribution

(metropolitan New York City area) can become so destructive that turfgrass managers may make multiple preventive applications of broad spectrum chemical insecticides throughout the growing season to avert aesthetic damage (McNeil et al. 1999). Insecticides are often applied to large areas of the course where the pest is not present in damaging densities. Consequently, the overuse of preventive chemicals has led to the development of pyrethroid resistant populations in some areas of the weevil's distribution (Cowles et al. 2008).

Listronotus maculicollis undergoes three generations per year in the metropolitan New York City area (McGraw and Koppenhöfer 2008a), yet the majority of damage to the turf is typically observed during the first larval generation and is often localized on the perimeters of fairways, greens, and tee boxes. Damage is particularly evident in short mown turfgrass stands with high percentages of annual bluegrass (*Poa annua* L.). Currently, the predominant management tactic is to preventively control egg laying adults as they migrate from overwintering sites to short mown areas in spring. Adults emerge from overwintering sites in the leaf litter and tall grasses in late March to mid April in New Jersey and over the course of several weeks walk on to short mown areas (less than 2.5 cm) where they feed and mate (Rothwell 2003; Diaz and Peck 2007; McGraw and Koppenhöfer 2007). Adult feeding may cause yellowing of the plant, but the vast majority of the damage occurs from larval feeding. Eggs are deposited directly into the stem of the turfgrass plant, and early stages (first through third instars) are stem borers. Most of the damage is caused by the fourth and fifth instar larvae tunneling through and feeding externally on the crown of the turfgrass plant. If left untreated, large populations

of late instar larvae may destroy turfgrass patches measuring several square meters to hundreds of square meters.

Sampling plans have yet to be developed for estimating *L. maculicollis* adult density or forecasting the potential threat of larval damage. Treatments are usually not based on population density but rather timed on plant phenological indicators or calendar dates (Tashiro et al. 1978). Furthermore, the relationship between adult and larval densities as well as those densities and damage are unclear, making the implementation of integrated pest management (IPM) or judicious use of curative controls difficult. The lack of efficient and reliable sampling protocols has intensified the reliance on preventive applications and left many gaps in our understanding of the pest's biology and ecology.

The objective of this study was to develop sequential sampling plans to classify adult density and forecast larval damage. Sequential sampling plans allow for efficient classifications or estimates of pest population densities, typically through flexible sample numbers (Binns et al. 2000). The advantages of sequential sampling for classification of pest density over extensive fixed sample number plans for estimation may be realized in reduced sampling time and cost (Hutchison 1994). To develop action thresholds and sequential sampling plans I sought to 1) determine the relationship between the density of larvae and turfgrass damage, 2) determine the relationship between adult and larval densities, 3) develop binomial (presence-absence) sequential sampling plans based on varying levels of risk, and 4) validate the plans using resampling software. The developed sampling plans should allow for more sustainable management of *L. maculicollis* populations.

Materials and Methods

Study Sites and design

Listronotus maculicollis populations were monitored on two golf courses in northern New Jersey with histories of *L. maculicollis* damage. Blocks were established on six separate fairways consisting of heterogenous mixtures of *P. annua* and creeping bentgrass, *Agrostis stolonifera* L. Three blocks were located at Brooklake Country Club (BLCC) (Florham Park, NJ) and three at Upper Montclair Country Club (UMCC) (Clifton, NJ). Previous research has demonstrated that emerging overwintered adults and first-generation larvae of *L. maculicollis* are aggregated and spatially associated along the edges of the fairway/rough borders (B. McGraw unpublished data). Therefore, blocks were arranged parallel to the edge of fairway/rough border adjacent to *L. maculicollis* overwintering sites. Each block contained 32 sample units (1.83 × 1.83 m grids), arranged 16 units parallel to the edge by two units into the the fairway (29.3 m length × 3.7 m width). The corners of blocks were permanently marked with plastic caps set flush with the soil surface to ensure the same sample units would be resampled in consecutive weeks. On each sampling occasion, monitoring blocks and sampling units were marked with corner flags. The time required for one person to set up and sample each block was recorded weekly and averaged across all sampling bouts.

Listronotus maculicollis adult sampling

Emerging overwintering adult *L. maculicollis* populations were monitored weekly between 2 April and 19 May 2007 and 31 March and 11 May 2008. The sampling period commenced 1 wk prior to when adults were expected to begin emerging from overwintering sites as estimated using traditional plant phenological indicators (*Forsythia*

spp.) (Tashiro et al. 1978). Adults were sampled weekly using a reverse air vacuum/leaf blower (Homelite Vac Attack II), fit with a mesh basket (324-mesh, 7.2 × 7.2 openings per cm^2) to capture weevils before entering the engine (Rothwell 2003). All blocks were sampled between 0600 and 0900 h. The order in which the blocks were sampled was randomized each week. Samples were taken sequentially from each block by vacuuming each sample unit for 10 seconds. The vacuum was moved across the surface of the turf, allowing for complete coverage of the sample unit during sampling time. After vacuuming the sample unit the basket was removed and contents emptied onto a tray to count the *L. maculicollis* adults. Adults were returned to the sample unit after counting. Weekly sampling ended when first instar larvae were no longer detected in plots adjacent to monitoring blocks, indicating that the emerging adults had ceased ovipositing (19 May 2007 and 11 May 2008). Between 2007 and 2008, 67 adult datasets were obtained for analyses.

Maximum and minimum air temperature data was used to transform dates from Julian date into degree days (DD) ranges for reporting *L. maculicollis* abundance in sampling intervals. Degree days were calculated using Arnold's (1960) degree day accumulations:

$$DD = (\text{maximum daily temperature} + \text{minimum}) / 2 - \text{Base temperature} \quad (6.1)$$

Degree days were accumulated beginning on 1 March using a 0°C base temperature (Rothwell 2003; Diaz 2006). Weather data was obtained from the National Oceanic and Atmospheric Administration (NOAA) website (www.noaa.gov) since onsite weather

stations proved to be inaccurate and were often missing data for extended periods. Weather monitoring stations were selected for proximity to golf courses, with the furthest station located 9.7 km away.

Listronotus maculicollis larval sampling

Listronotus maculicollis larvae were sampled on 28 May 2007 and 19 May 2008. Six soil cores (5.4 cm diameter × 5 cm depth) were removed from each sample unit using a Turf Tec International soil corer (Oakland Park, FL). Soil samples were placed in a polyethylene bag, sealed, and transported to the laboratory in a cooler. In 2007, larvae were manually extracted from soil cores, followed by extraction of larvae from the plant material by submersion in a saturated saline solution. In 2008, larvae were extracted by placing the soil cores on Berlese funnels in incubators at 29–32°C for 7 d followed by manual examination of the plant material, thatch, and soil. This allowed for a more efficient sampling of younger and older larvae (Diaz 2006; B. McGraw unpublished data). The stage composition of the larval populations was determined by measuring the width of the head capsule of larvae in a random subset of samples according to Cameron and Johnson (1971). In 2008, head capsules measurements were taken from larvae in samples manually extracted, since heat extraction would cause stages to advance beyond the stage at the time of sampling. The larval population was evenly distributed between second through fourth instars in 2007. In 2008, the population was sampled 1 wk earlier than 2007 and had a more variable distribution and a greater percentage of younger larvae (5, 54, 36, and 5% first instars through fourth instar, respectively).

Pearson's correlations were performed between larval densities and adult counts by sampling date and cumulative counts within a block using Statistix 8.0 (Analytical

Software 2003). Data from 2007 and 2008 were analyzed separately since the methods for estimating larvae within sample units differed between years. When positive significant relationships were found between adult counts and larval densities, linear regression analyses were performed to generate equations to relate the two variables.

Turfgrass damage

Turfgrass species within each sample unit were identified on 13 April 2008 to determine the relationship between larval density and turfgrass species on damage expression. A 30.5 cm × 30.5 cm frame, divided into 25 sub-squares with monofilament line, was randomly tossed within each sample unit and the dominant turfgrass species within each sub-square was identified and recorded. Damage assessments were made on 23 June 2008 using the same randomized sampling methodology as described above. Damage was considered to be significant yellowing of the grass plants within the sub-squares. The number of sub-squares damaged was divided by the total number of squares in the frame to determine the percent damaged. A damage model based on the density of *L. maculicollis* larvae and composition of turfgrasses within each sample unit was developed using multiple linear regression analyses.

Binomial sampling plans

Eight binomial (presence-absence) sequential sampling plans were constructed based on four adult density thresholds and two tally thresholds for assigning infestation. The four adult density thresholds (0.5, 0.85, 1.15 and 1.35 average adults per sample unit) were arbitrarily chosen based on field observations of damage occurring in blocks where average adult density exceeded one adult per sampling unit (B. McGraw, unpublished data). Therefore, the four critical densities represent a range from strict (lowered risk to

damage) to relaxed approaches (higher risk to damage) to managing *L. maculicollis* populations. Two low tally thresholds (T = 1 and 2 adults) were chosen since *L. maculicollis* adults occur in relatively low counts at the scale of the sampling unit (B. McGraw unpublished data). Also, increasing the tally threshold has been shown to decrease the variability and increase the precision of density estimates (Binns et al. 2000). To develop sampling plans based on binomial counts, the relationship between the adult density and proportion of infested sampling units (PI) for each tally threshold was established through non-linear regression. Both tally thresholds produced strong relationships between the mean density of adults and the PI (r^2 = 0.92 and 0.96 for T = 1 and T = 2, respectively) (Fig. 6.1). Regression equations were used to transform adult densities into four action thresholds or critical proportions infested (CP) for each tally threshold (T = 1: 0.33, 0.52, 0.65, 0.74 PI; T = 2: 0.12, 0.21, 0.29, 0.34 PI).

Resampling for Validation of Sample Plans (RVSP) software was used to resample the field collected datasets to generate operating characteristic (OC) curves, average sample numbers (ASN), and sampling plan stop-lines (Naranjo and Hutchison 1997). Resampling was performed using 500 iterative runs (conducted with replacement) using a minimum sample size of 10 sample units. Operating characteristic functions plot the probability of not taking action against pest densities with increasing proportion of infested sample units and can be considered an estimate of the precision of the sampling plan (Binns and Nyrop 1992).

Decision stop lines were developed based on Wald Sequential Probability Ratio Test (SPRT) (Wald 1947). Developing stop lines for Wald's SPRT requires the CP, upper and lower boundaries around the CP (θ_1 and θ_0, respectively) and levels of risk

associated with Type I and Type II error rates. Type I (no treatment is made when a treatment is required) and Type II errors (treatment is made when no treatment is required) were set equal to each other for all plans (0.1). Boundaries θ_1 and θ_0 were arbitrarily set 0.1 higher and lower than the CP in all plans. The upper (UL) and lower (LL) boundaries or stop lines and their common slope was calculated as follows:

$$UL = \ln(1-\beta/\alpha) / \ln(\theta_1 q_0/\theta_0 q_1) \quad (6.2)$$

$$LL = \ln(\beta/1-\alpha) / \ln(\theta_1 q_0/ \theta_0 q_1) \quad (6.3)$$

$$Slope = \ln (q_0/q_1) / \ln(\theta_1 q_0/ \theta_0 q_1) \quad (6.4)$$

where α is the Type I error rate, β is the Type II error rate, $q_1 = 1- \theta_1$, and $q_0 = 1- \theta_0$.

Sampling plans were evaluated to determine the optimal tally threshold and CP combination for each adult density threshold through several methods. First, the conservativeness of the sampling plan was estimated by examining the OC values when adult densities were at the CP (Hodgson et al. 2004). Operative characteristic values greater than 0.50 at the CP suggest that the plan is liberal or that no action is taken more than half of the time when action should be taken. A conservative plan, one where action is taken more often than needed, would have OC values less than 0.50 around the CP. An ideal sampling plan theoretically would be neither conservative nor liberal when values are estimated around the CP (equaling 0.50). Secondly, four cell probability matrices were constructed to evaluate the proportion of correct management decisions generated

by each of the eight binomial sequential sampling plans (Burkness et al. 1999). Four outcomes can occur after the cumulative number of adults has crossed an upper or lower stop-line: a correct decision to take action to treat (A), an incorrect decision to treat (B), an incorrect decision not to treat (C), or a correct decision not to treat (D). The sum of A+D equals the proportion of correct decisions and the sum of B+C equals the proportion of incorrect decisions. Operating characteristic functions from resampling the datasets were used to determine the proportion of 500 simulation runs for each dataset resulted in a "treat" or "no treat" decision. The actual PI from the dataset was compared to simulated PI to determine whether the decision was correct. The total number of decisions was summed across all of the datasets and divided by the total number of simulations for all datasets (33,500).

The sampling plans were directly compared through benefit-to-cost ratio (BCR) analysis (Burkness et al. 1999). The BCR is determined by the proportion of correct decisions (A+D) and the cost of obtaining the PI. It is calculated as follows:

$$BCR = [\sum P(A+D) / ((ASN \times C_1) + C_2)] \times 100 \quad (6.5)$$

where $\sum P(A+D)$ is the summed probability of correct decisions, ASN is the average number of samples required to classify density, C_1 is the cost or time required to measure and flag the monitoring block, and C_2 is the time required to take one sample. The ASN for each CP and tally threshold was determined through resampling simulation. The average time to set up the monitoring blocks (C_1 = 10.4 +/- 4.9 min standard deviation) and the time required to sample an entire block (22.3 +/- 3.9 min) was averaged across all

weeks and years (data not shown). The time required to sample an entire block was divided by the number of samples in the block (n = 32) to estimate cost of taking one sample (C_2 = 0.70 min/sample). The time to sample one unit was considered to be the same for each tally threshold.

Results

Adult counts and larval density

Variability was observed in the seasonal dynamics of adult captures on fairway edges between sites and years (Fig. 6.2). Adult densities peaked on fairway edges on two different sampling dates, a phenomenon observed in previous *L. maculicollis* population studies (Diaz 2006). The timing of the peaks was consistent in each of the two years of the study (21–23 April = 331–424 DD, 5–7 May = 528–615 DD). In 2007, these peaks occurred between 331 and 391 DD (23 April) at UMCC and between 528 and 599 DD (7 May) at BLCC. The following year, the seasonal phenology was more consistent between sites. Five of the six blocks had the highest adult densities on the second peak (5 May; between 564 and 615 DD) in 2008.

Strong significant relationships were found between adult counts and future larval densities within sampling units (Table 6.1). Adult counts during the second peak of abundance (528–599 DD in 2007 and 564–615 DD in 2008) and cumulative adult counts across the study were consistently correlated to larval densities within sampling units in both years of the study. The correlations suggest that oviposition occurs over an extended period of time, yet the majority of eggs are deposited during the second peak in abundance. Therefore, to reduce sampling costs and develop practical monitoring

protocols, sampling may be reduced to the periods surrounding the second peak in adult abundance (498 to 615 DD in this study).

Larval density and damage

A strong significant relationship between larval density (x_1) and the proportion of damaged turfgrass (y) was detected through linear regression ($F = 131.4$; df = 1, 131; $P < 0.0001$; $y = -0.016 + 0.001\ x_1$). The model indicates that 120 larvae per 0.09 m^2 would produce 10% turf loss, a value arbitrarily set by turfgrass managers as the threshold for visible damage (B. Dickison personal communication). Multiple linear regression analyses revealed a highly significant model describing turfgrass damage as a function of larval density (x_1) and the proportion of *P. annua* (x_2) in sample units ($F = 81.7$; df = 2, 131; $P < 0.0001$; $y = -0.042 + 0.0008\ x_1 + 0.351\ x_2$). A damage matrix was constructed for larval density and turfgrass species composition (Table 6.2). The model predicts that as little as 10 larvae per 0.09 m^2 is capable of causing visible damage in plots with 40% *P. annua*, yet in pure *A. stolonifera* plots 160 larvae are required before damage is visible.

Binomial sampling plans

Operating characteristic functions were plotted for each of the eight sampling plans (Fig. 6.3A–H). Six of the 8 plans were conservative when the actual proportion infested neared the CP (OC < 0.50), indicating that treatment decisions were made more than half of the time when no treatment was required. Increasing the tally threshold from one to two adults per sampling unit resulted in all four plans becoming conservative when densities approached the CP. One plan (T = 2, CP = 0.34) produced a near optimum curve when the actual proportion of infested sampling units neared the CP (OC of 0.48).

Two plans produced slightly liberal OC curves at the CP (Fig. 6.3B and D; OC = 0.55 and 0.56, respectively). Two of the three plans with near optimal OC curves at the CP had the highest adult density thresholds (Fig. 6.3D and H).

The four cell probability matrices demonstrate that correct "treat" and "no-treat" decisions (A+D) were made more than 86% of time in all sampling plans (Table 6.3). Correct decisions increased with an increase in CP within both tally thresholds. Incorrect decisions to withhold treatment when treatment was required (B), representing the worst case scenario, were made less than 5% of the time, with the exception of the lowest adult action density threshold at T = 2 (7%). Increasing the tally threshold did not produce a discernable pattern to incorrect decisions.

The ASN was considerably lower than the maximum number of sample units in the block for all sampling plans (Table 6.3; 11.4 to 17.6 samples). Resampling analysis suggests that the costs associated with the plans are relatively inexpensive, averaging 22.7 minutes or less to arrive at a decision. However, the number of samples needed to classify density when the actual proportion infested neared the CP exceeded the maximum sample number in the block in two of the 8 plans (Fig. 6.4). Increasing the tally threshold produced mixed effects on the ASN. For the lower, more conservative adult action density threshold (0.5 and 0.85 adults per sampling unit), ASN decreased with an increase in tally threshold. However, the opposite was true for the more relaxed adult density thresholds (1.15 and 1.35 adults per sampling unit).

Benefit to cost analysis revealed that increasing the CP for T = 1 increased the benefit of the sampling plan (Table 6.4). For T = 1, the increase in CP caused the ASN to decrease and the probability of making a correct decision to increase, thus increasing the

BCR. However, for T = 2, the increase of the BCR with CP could be attributed solely to the increase in the probability of making correct decisions. The lower tally threshold produced higher BCR for the sampling plans with lower adult density thresholds, whereas the higher adult thresholds were improved by the increased tally threshold.

Stop-lines

Four binomial sampling plans stop-lines, one for each adult density threshold, are presented since the thresholds represent a range of management scenarios (Fig. 6.5). Sampling plans were selected for the optimal tally threshold and CP combinations for each adult density based largely on BCR and the probability of making correct decisions. Three of the four plans selected require the use of the lower tally threshold, despite the improvement in BCR for an increased tally threshold in two of the four plans. The lowest adult threshold (0.5 adults) produced a lower ASN and higher BCR with a T = 2 than with T = 1. However, incorrect decisions were made 14% of the time (7% of which were "incorrect no treatments") compared to 8% for T = 1. Adopting this sampling plan, though likely to be more accurate, would require approximately 5.5 more samples on average (3.85 min). Near optimal OC curves were obtained for the adult threshold 1.35 using T = 2 when analyzed at the CP (OC = 0.48), yet the T = 1 plan was chosen since it produced lower ASN and higher BCR and probabilities of correct decisions.

Discussion

This study provides efficient and reliable binomial sequential sampling plans to forecast *L. maculicollis* larval densities and assess the threat of damage. Previously, the relationship between adults and larvae, larval thresholds for damage, and the sampling

protocols had not been explored for *L. maculicollis*, which has hindered the implementation of IPM programs involving curative and alternative controls. The evaluation of the sampling plan stop-lines or management scenarios suggests that binomial sampling plans have a high probability for accurately classifying *L. maculicollis* densities with minimal cost.

Visible turfgrass damage from first generation larvae typically occurs on the edges of short mown areas (Vittum et al. 1999). The damage is most evident in turfgrass stands where the composition of grasses is predominately *P. annua*. These observations have led turfgrass managers and researchers to make assumptions of host preference largely supported by laboratory and small plot choice and no-choice experiments (Rothwell 2003). The data suggest that not only larval density, but the composition of turfgrasses greatly affect the expression of damage. The findings presented here in combination with studies of non-manipulated field populations (B. McGraw unpublished data) provide some evidence that *L. maculicollis* may not be preferentially selecting *P. annua* over *Agrostis* spp. but rather damage is expressed at lower densities in *P. annua* giving the appearance of host preference or selectivity.

Vittum (2005) proposed first generation late instar larval thresholds between 30 and 80 larvae per 0.09 m^2. The data presented in this study suggest that these thresholds are appropriate for most larval densities and turfgrass species mixtures. However, these damage thresholds may be too high in mixed fairways with high percentages of *P. annua* and too low for pure *A. stolonifera* fairways. However, caution should be employed in adjusting current damage thresholds, especially when applying lower thresholds than currently recommended. The larval estimates in this study are based on sampling

younger larvae and therefore may overestimate the number of insects that reach the damaging stages given natural and predator induced mortality (Vittum 1980; Rothwell 2003; Diaz 2006; McGraw and Koppenhöfer, 2008a). Most importantly, the findings presented here indicate that *A. stolonifera* is more tolerant to *L. maculicollis* feeding than *P. annua*. *Listronotus maculicollis* has been shown to oviposit fewer eggs in *A. stolonifera* and develop slower when compared to *P. annua* (Rothwell 2003). Taken together these data suggest that removing *P. annua* and re-seeding more tolerant turfgrass species may be a sustainable management tactic to suppress *L. maculicollis* populations or at least avoid aesthetic damage while reducing chemical inputs.

The mathematical relationship between adult density and the proportion of sampling units infested allowed for the development of binomial sampling plans for *L. maculicollis*. The decision to use flexible sample size binomial sampling plans over fixed sample sized plans or enumerative plans based on precision estimates was an issue of practicality. Binomial sampling plans can greatly reduce sampling costs by recording only the presence of the pest. This has been shown to provide great benefits to sampling pests that occur in high densities (Wright and Cone 1999; Opit et al. 2003; Hodgson et al. 2004). Sampling time can be greatly reduced for highly aggregated pests when only presence is recorded rather than full counts. Conversely, presence-absence plans have been shown to deliver quick and accurate classification of pests that occur in low density or are only weakly aggregated (Galvan et al. 2007). *Listronotus maculicollis* adults demonstrate a low level of aggregation along the edges of fairways (B. McGraw., unpublished data) and are found in low densities at the scale of our sampling units. Therefore the benefits of reduced time per sample unit are not realized, but rather the

ability to obtain reliable classifications of densities using a relatively small total sample size (32 samples). Since many different locations on a golf course are likely to have areas prone to *L. maculicollis* damage, sampling plans must allow for areas to be sampled reliably yet quickly so that other areas may be sampled. Therefore, monitoring blocks were designed based on the maximum time that would be practical for turfgrass managers to spend in one area if the total number of samples were to be taken (32.8 min). Resampling analysis showed that on average the sampling plans were capable of arriving at a decision in approximately 20 min, which included time to measure and mark the monitoring blocks. The information lost by using binomial sampling plans to classify the infestation (estimation of the mean) is more likely to be important to ecologists rather than pest managers.

A maximum sample number was placed on sampling plan stop-lines for two purposes. First, the blocks were designed and marked to allow for re-sampling the same sample units in consecutive weeks. This allows for the turfgrass managers to accurately monitor the same area over time. Secondly, without a maximum sample size the cumulative number of adults could indefinitely be located between the sampling plan's stop-lines. After the minimum number of sampling units has been taken, the sampler is faced with four options based on the cumulative number of adult captured: treat, continue sampling, stop sampling and resample later, or stop sampling and do not treat. Given the correlation between adult counts and larval densities during the second peak in adult abundance, sampling should be initiated prior to the first week of May or prior to 500 DD in the New York metropolitan area. Adult estimates falling below the sampling plan stop-line or remaining between stop-lines after the maximum number of samples is taken

should be resampled at a later date. The frequency and timing of resampling will be dependent on costs (labor and time), the number of sites requiring sampling, and the level of risk to damage that the operation is willing to take.

There are a great number of barriers to developing IPM programs on golf courses given low tolerances for aesthetic damage and the reliance on preventive synthetic insecticides. However, the development of accurate and rapid monitoring programs should allow for the incorporation of more curative and alternative control strategies that minimize unnecessary interventions in time and space. The success of the sampling plans developed for *L. maculicollis* will be dependent on further evaluation in other areas of the weevil's distribution, and therefore should be viewed as initial attempts to aid in monitoring programs for *L. maculicollis*. Further research is needed to develop accurate methods for timing of larval stages across all areas within the weevil's distribution to be combined with the sampling plans proposed in this study to optimize targeted curative controls.

Table 6.1. Linear regression analysis of adult counts (y) by sampling date and future larval densities (x) in 3.34 m^2 sampling units.

Year	Sampling Date	Degree Days[a]	Correlation coefficient	*F*	*P*	Regression equation[b]
2007	2 April	185–214	-0.071		0.325	
	9 April	220–249	0.185	6.74	0.01	$y = 0.0284 + 0.00163\ x$
	16 April	257–298	*n/a*		*n/a*	
	23 April	331–391	-0.023		0.756	
	30 April	433–498	0.119		0.1	
	7 May	528–599	0.216	9.28	0.003	$y = 0.353 + 0.00403\ x$
	14 May	649–722	0.013		0.862	
	21 May	763–843	0.166	5.37	0.022	$y = 0.226 + 0.00258\ x$
	cumulative	185–843	0.196	7.62	0.006	$y = 1.70 + 0.0103\ x$
2008	31 March	158–171	-0.037		0.608	
	7 April	218–236	-0.085		0.24	
	14 April	298–319	0.005		0.948	
	21 April	387–424	0.066		0.361	
	28 April	482–531	0.273	15.30	< 0.0001	$y = 0.0921 + 0.000801\ x$
	5 May	564–615	0.310	20.16	< 0.0001	$y = 1.30 + 0.00420\ x$
	12 May	658–721	0.293	17.90	< 0.0001	$y = 0.237 + 0.00153\ x$
	cumulative	158–721	0.382	32.37	< 0.0001	$y = 2.49 + 0.00681\ x$

[a] Degree days were accumulated starting 1 March using 0°C base temperature. The range of degree days by dates accounts for the difference in accumulated temperature between golf courses in the study.

[b] Regression equations are provided only for significant relationships between adult counts and larval densities in 3.34m^2 sample units.

Table 6.2. The proportion of damaged turfgrass (y) as a function of *Listronotus maculicollis* larval density (x_1) and the proportion of *Poa annua* (x_2) in 3.34 m^2 sample units based on the model $y = -1.05 + 0.0208\ (x_1) + 8.77\ (x_2)$ ($F = 81.7$, df = 2, $P < 0.0001$). Bold and underlined values represent the combination of larval density and *P. annua* that cause around 10% turfgrass damage, or the minimum density causing visible damage.

	Density of *L. maculicollis* larvae (per 0.09m^2)															
P. annua	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160
0.00						0.01	0.01	0.02	0.03	0.04	0.05	0.05	0.06	0.07	0.08	**0.10**
0.10		0.01	0.02	0.03	0.03	0.04	0.05	0.06	0.07	0.07	0.08	**0.10**	**0.10**	**0.11**	**0.11**	0.12
0.20	0.04	0.04	0.05	0.06	0.07	0.08	0.08	**0.09**	**0.10**	**0.11**	**0.12**	0.12	0.13	0.14	0.15	0.16
0.30	0.07	0.08	**0.09**	**0.10**	**0.10**	**0.11**	**0.12**	0.13	0.14	0.14	0.15	0.16	0.17	0.18	0.18	0.19
0.40	**0.11**	**0.11**	0.12	0.13	0.14	0.15	0.15	0.16	0.17	0.18	0.19	0.19	0.20	0.21	0.22	0.23
0.50	0.14	0.15	0.16	0.17	0.17	0.18	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.25	0.25	0.26
0.60	0.18	0.18	0.19	0.20	0.21	0.22	0.22	0.23	0.24	0.25	0.26	0.26	0.27	0.28	0.29	0.30
0.70	0.21	0.22	0.23	0.24	0.24	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32	0.32	0.33
0.80	0.25	0.25	0.26	0.27	0.28	0.29	0.29	0.30	0.31	0.32	0.33	0.33	0.34	0.35	0.36	0.37
0.90	0.28	0.29	0.30	0.31	0.31	0.32	0.33	0.34	0.35	0.35	0.36	0.37	0.38	0.39	0.39	0.40
1.00	0.32	0.33	0.33	0.34	0.35	0.36	0.37	0.37	0.38	0.39	0.40	0.41	0.41	0.42	0.43	0.44

Table 6.3. Comparison of the eight binomial sequential sampling plans developed for classifying *Listronotus maculicollis* adult densities based on the probability of correct (∑P (A+D)) and incorrect (∑P (B+C)) treatment decisions, average sample number (ASN), and cost and benefit-to-cost ratio (BCR).

Tally threshold	Adult action threshold	Critical Proportion[a]	Correct Treat	Incorrect No Treat	Incorrect Treat	Correct No Treat	Correct Decisions	Incorrect Decisions			
(T)	(Mean)	(CP)	(A)	(B)	(C)	(D)	∑P (A+D)	∑P (B+C)	ASN[b]	Cost[c]	BCR[d]
1	0.5	0.33	0.29	0.05	0.03	0.64	0.92	0.08	17.6	22.70	120.2
	0.85	0.52	0.11	0.04	0.02	0.82	0.94	0.07	15.6	21.35	129.2
	1.15	0.65	0.05	0.02	0.01	0.92	0.97	0.03	12.8	19.35	147.8
	1.35	0.74	0.02	0.02	0.00	0.96	0.98	0.02	11.4	18.38	158.4
2	0.5	0.12	0.24	0.07	0.07	0.62	0.86	0.14	12.1	18.88	133.6
	0.85	0.21	0.11	0.03	0.03	0.84	0.95	0.05	13.9	20.11	137.4
	1.15	0.29	0.05	0.02	0.01	0.92	0.97	0.03	13.6	19.90	141.8
	1.35	0.34	0.02	0.03	0.00	0.95	0.97	0.03	13.0	19.49	145.1

[a] Critical Proportion (CP) is the adult action threshold transformed into the proportion of sample units infested through the non-linear regression.

[b] Average sample number (ASN), the number of samples required to make a sampling decision, calculated by Resampling for Validation of Sampling Plans (PVSP) software using 500 iterative sampling runs of field datasets.

[c] The cost of sampling (in minutes): $C = C_1 + (ASN \times C_2)$, where C_1 is the average time required to set-up the sampling block (10.4 +/- 4.9 min standard deviation), C_2 is the average time required to take one sample, calculated as the time to take samples from the entire block (22.3 +/- 3.9 min) divided by total number of samples taken (32) = 0.70 min/sample).

[d] Benefit-cost-ratio (BCR): $[\sum P(A+D) / ((ASN \times C_1) + C_2)] \times 100$.

Figure 6.1. Non-linear relationship between mean density of *Listronotus maculicollis* adults and the proportion of sample units infested with one (T = 1) or two (T = 2) adults. The monitoring blocks contained 32 3.34 m^2 sample units.

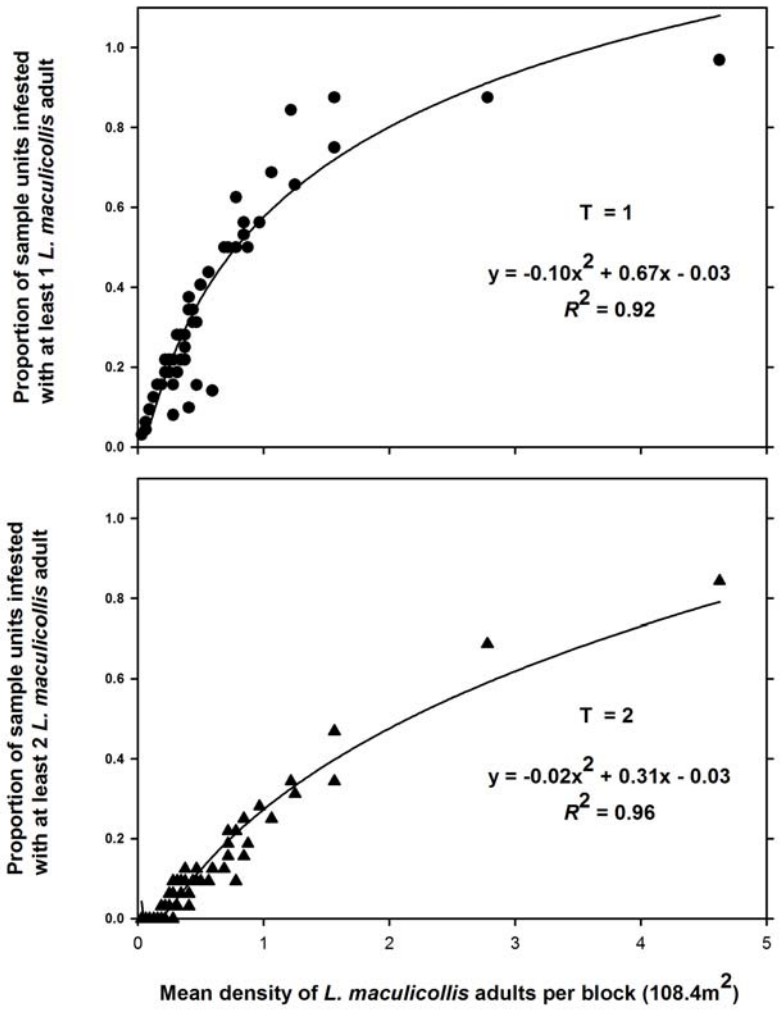


Figure 6.2. Seasonal variation in abundance of emerging *Listronotus maculicollis* adults in monitoring blocks at Upper Montclair Country Club (UMCC) and Brooklake Country Club (BLCC). Densities were estimated from vacuum sampling of 3.34 m^2 sample units from emergence through oviposition in 2007 and 2008.

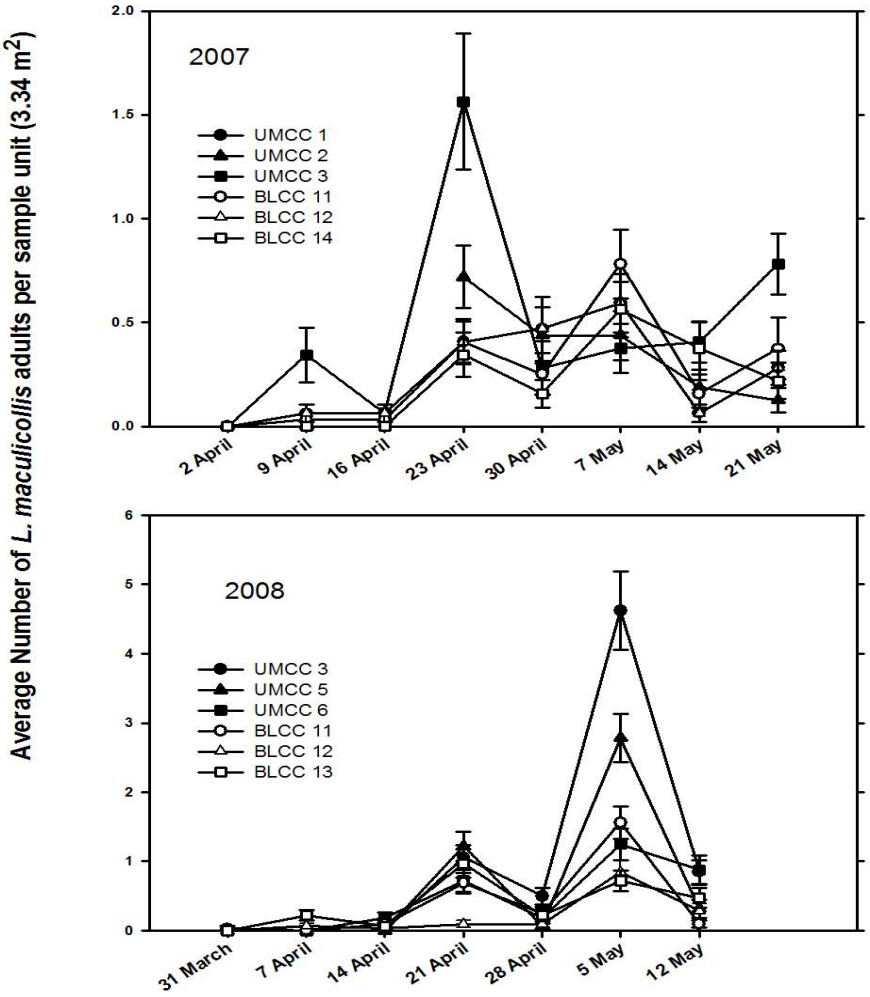


Figure 6.3. Operating characteristic (OC) functions for binomial sequential sampling plans for *Listronotus maculicollis* adults. The 8 plans represent two tally thresholds for classifying infestation (T = 1, 2) and four adult density thresholds (0.5, 0.85, 1.15, and 1.35 adults per sample unit). The OC function generated through resampling software was plotted against the observed (circles) and simulated (solid line) proportion of infested sample units.

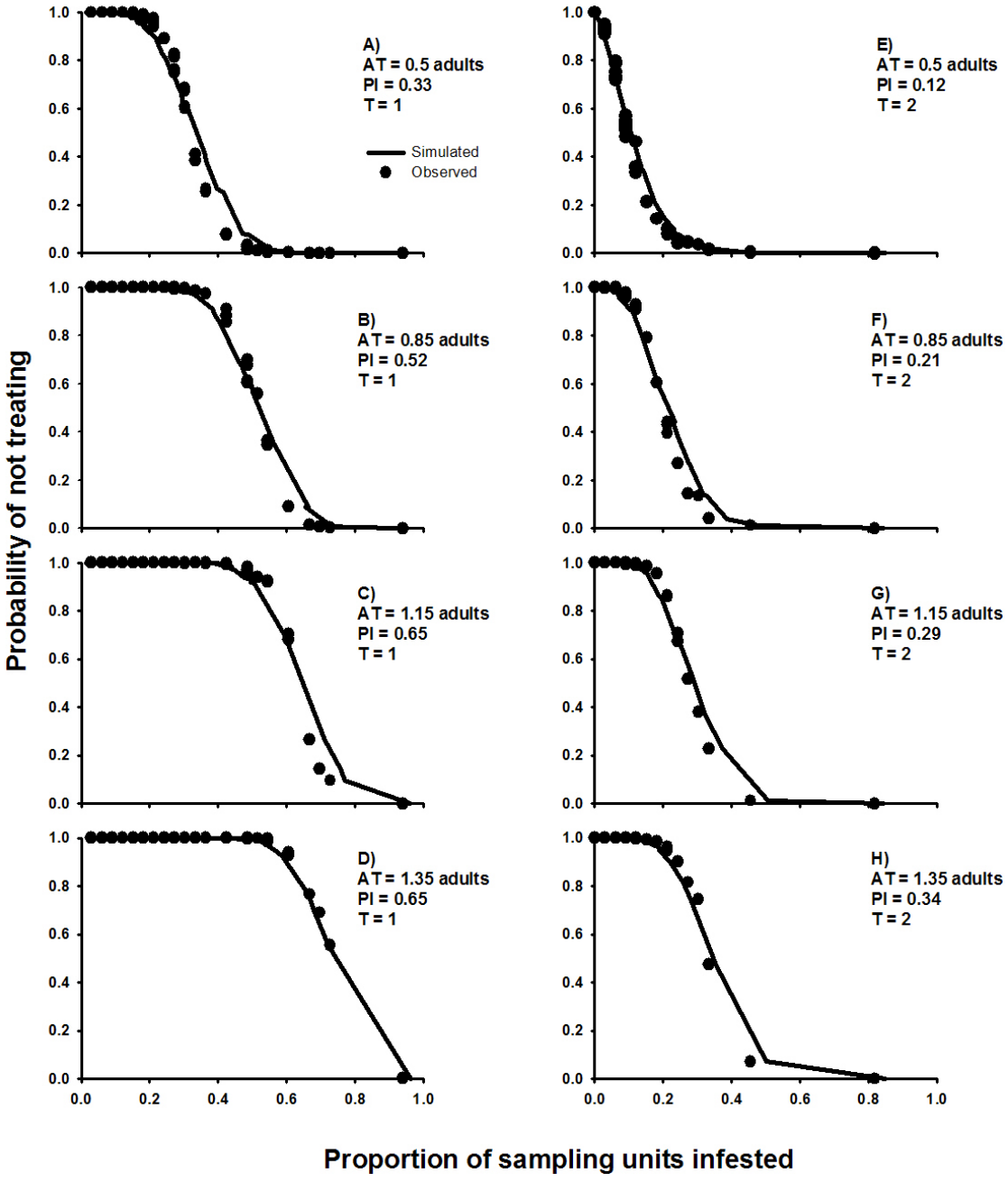


Figure 6.4. Average sample number (ASN) for binomial sequential sampling plans based on Wald's SPRT for a tally threshold of one adult (A) and two adults (B).

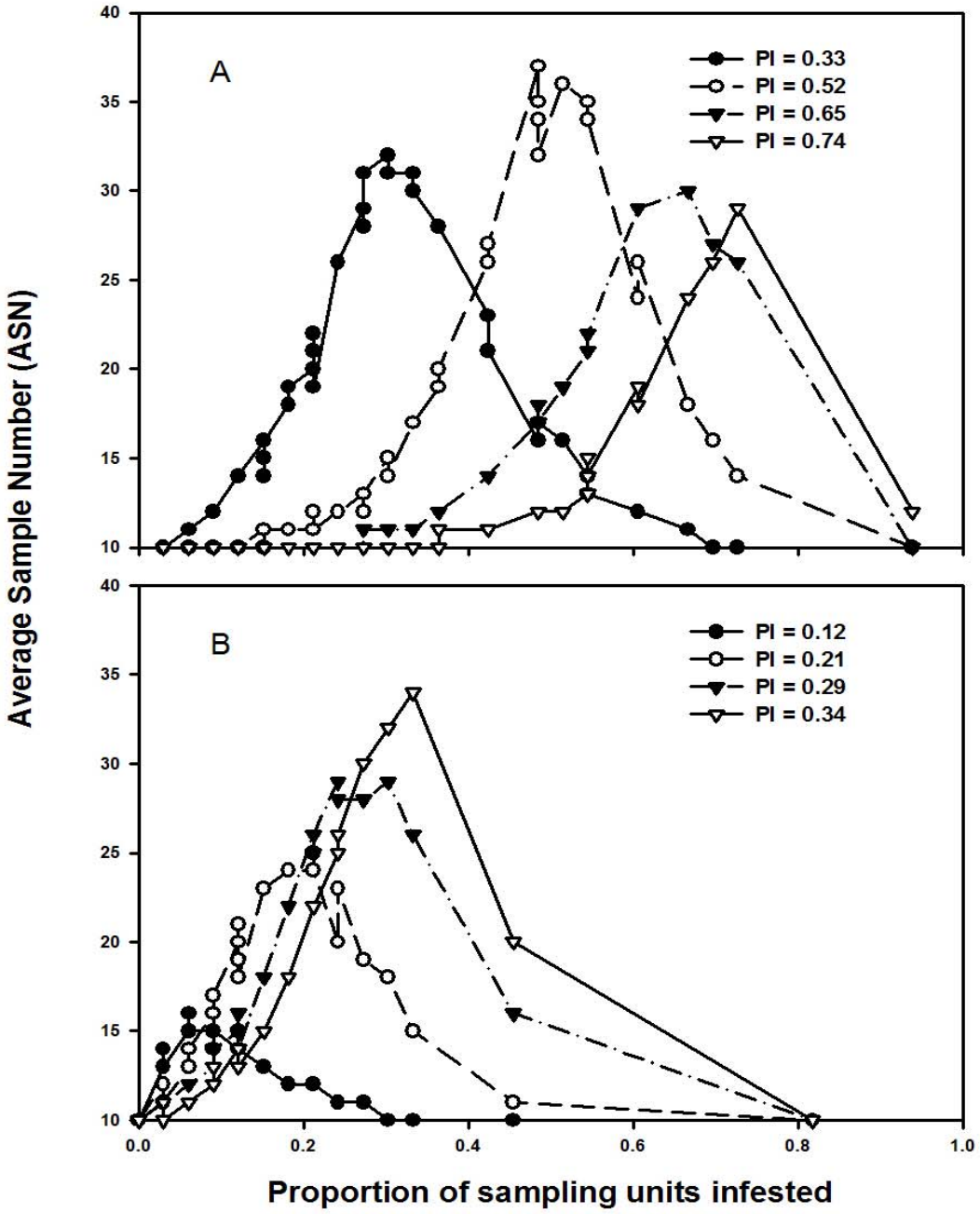
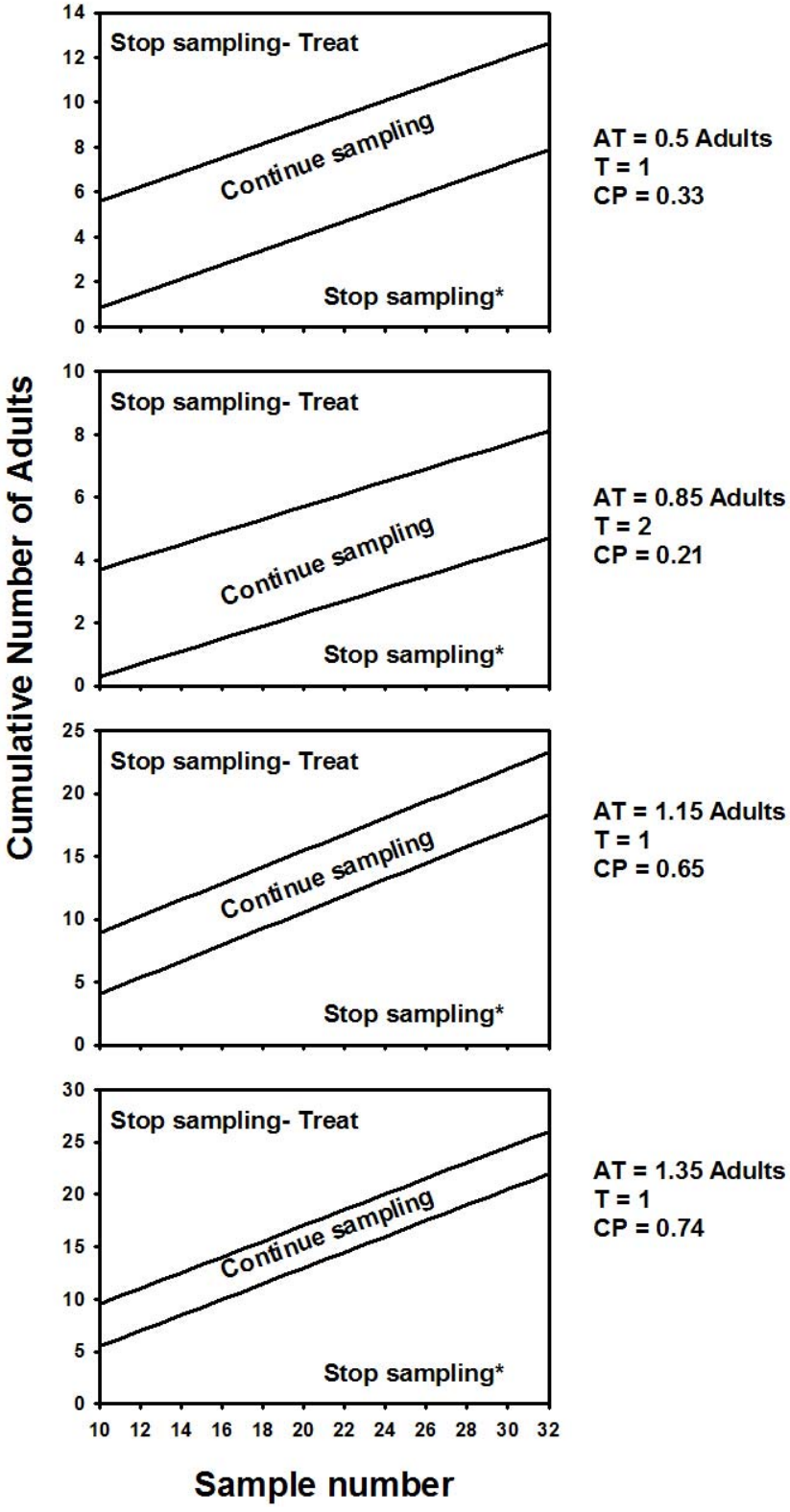


Figure 6.5. Binomial sequential sampling decision stop-lines based on Wald's SPRT. Each of the four plans represents the optimal tally threshold and critical proportion combination for the given adult density threshold.



CHAPTER SEVEN

CONCLUSION

There are many obstacles to developing biological control programs for pests of golf course and fine turfgrass. The low tolerance for damage and the availability of cheap chemical pesticides has created a pest management culture that is unable to determine where and when intervention is warranted. The long history of preventive pest management in these systems has created scenarios were chemical pesticides are abused in both time and space, which in the case of *L. maculicollis* has caused the development of insecticide resistant populations. I sought to explore the use of entomopathogenic nematodes for the biological control of *L. maculicollis* in anticipation of insecticide resistance developing in New Jersey and to develop more ecologically sensitive management strategies for controlling weevil populations. The seasonal ecology of the weevil was studied to understand the variability in control by entomopathogenic nematodes and to effectively integrate controls. The thesis is presented in chronological order of the research, and hopefully demonstrates the thought process involved in developing a biological control program.

Entomopathogenic nematodes have long been used in augmentative biological control programs, yet little is known about their field ecology or the potential of endemic nematode populations to reduce pest populations. A greater understanding of the dynamics of field populations of pathogens and their impact on pest species should help in the development of biologically based management programs. *Steinernema*

carpocapsae and *H. bacteriophora* were isolated from epizootics in *L. maculicollis* populations in an initial survey for natural enemies and pathogens. Both nematodes were found naturally infecting a majority of *L. maculicollis* stages and caused moderate reductions in host populations. However, the sensitivity of nematode populations to environmental conditions and variability in generational mortality suggest that endemic populations alone cannot reliably reduce *L. maculicollis* on golf courses given the low tolerance for aesthetic damage. Therefore, investigations into augmenting nematode populations through inoculative or inundative releases were conducted.

Laboratory bioassays were developed to screen commercially available species and strains isolated from infected *L. maculicollis* cadavers to different stages of the weevil. Differences were observed in the susceptibility to nematode infection as the insect aged. Several nematode species were able to cause significant mortality to *L. maculicollis* fourth- and fifth-instar larvae in naturally infested turf/soil plugs under controlled conditions, particularly *S. feltiae* and *S. carpocapsae.* Nematode isolates obtained nematode-infected *L. maculicollis* cadavers recovered from New Jersey golf courses were not more virulent than the commercial species to any stage tested. The tested species/strains of nematodes were not sufficiently virulent to *L. maculicollis* adults and are unlikely to be a viable option for replacing synthetic chemical insecticides in a preventive management program on any golf course. The decrease in susceptibility to infection as the weevil ages, especially between larval instars indicates that timing of nematode applications in the field will be crucial for effective control.

Field evaluations of entomopathogenic nematodes against first-generation larvae confirmed results obtained in laboratory bioassays, including the effect of nematode

species and strains. Control levels were generally lower than levels observed in the laboratory, as could be expected with less than optimal environmental conditions. Great variability was observed in the efficacy of nematode treatments at standard field application concentrations, which made detection of statistically signicant differences difficult. Thus, nematodes often provided large reductions in *L. maculicollis* larval densities when applications were timed to coincide with larval stages entering the soil (fourth instars), especially *S. feltiae* (94%). Overall, *S. carpocapsae* performed the most consistently between years, but failed to provide greater than 70% control. Variable control between years was believed to be due to a combination of factors, including the ratio of nematodes to weevils, timing of applications relative to the distribution of the population and an inability of nematodes to persist for significant periods of time. Field persistence of nematodes was observed to be low, dramatically declining between application and 14 DAT. Minimal recycling was detected after 28 DAT in most treatments, although all treatments were practically undetectable in the period when second-generation weevil larvae entered the soil.

The failure of entomopathogenic nematodes to provide significant control led to an investigation into the spatial variability of first-generation *L. maculicollis* larvae. The dispersal and foraging behaviors of emerging populations of *L. maculicollis* adults were examined between emergence from overwintering sites through the oviposition period to develop monitoring programs to predict the severity of potential damage, and to determine the potential of spatially targeted controls. Spatial analyses of adult populations indicated progressive movement through the edges of the fairway-rough border, leading to a significantly aggregated distribution of cumulative captures. The

distribution of cumulative adults captures was significantly spatially associated with the distribution of future larvae, suggesting that the majority of eggs are deposited as adults arrive on the margins of fairways over the course of several weeks. Surprisingly, the spatial distribution of *Poa annua*, the believed preferred host plant of *L. maculicollis*, did not affect the distribution of either *L. maculicollis* stage. Instead, the larval aggregations occurring along the edges of fairways suggest a preference for short mown hosts over long, and that the placement of eggs is driven by a low encounter rate with short mown hosts, rather than preference for species or cultivar.

Incorporating biological controls or even judicious use of synthetic chemical insecticides will require detailed information on where the pest is present, and whether the densities warrant intervention. Results obtained from the spatial studies of *L. maculicollis* were used to develop binomial sequential sampling plans to forecast the potential threat of larval feeding damage. Monitoring blocks were designed to sample adult populations as they emerged on to the edges of fairways. Counts of adults on the edges of fairways during peak emergence were positively correlated to the densities of future larvae. Four binomial sequential sampling plans were developed to allow turfgrass managers to determine their operation's level of risk of damage and make decisions accordingly. After a minimum of ten samples are taken, the density of adults is estimated. This allows for a flexible number of samples to be taken, and potentially can reduce the cost of sampling compared to extensive full-count sampling.

A highly significant effect of turfgrass composition and larval density was observed on the expression of turfgrass damage in small field plots. A damage model was constructed from multiple regression analyses. It suggests that plots comprised of

purely *Agrostis stolonifera* require 15-fold higher larval densities than *P. annua* before damage is expressed. This finding is further supported by the results from the *L. maculicollis* and *P. annua* spatial association studies. Furthermore, the data suggest that differences in the tolerance to larval feeding between turfgrass species may give the appearance of host preference.

The findings of this thesis suggest that entomopathogenic nematodes are capable of reducing *L. maculicollis* larval populations on golf courses. However, the variability in weevil populations and level of suppression by currently available nematode products make nematodes unlikely to be adopted by any golf course. Although the nematodes tested did not provide consistent control, the findings suggest that there may be a potential for spatially targeting controls should more virulent or specific species or strains be uncovered. Future research will be needed to address some of the sources of variability in control if entomopathogenic nematodes are to be effectively used in golf course turfgrass pest management. It is my hope that the research presented in this thesis will aid in our understanding of endemic entomopathogenic nematode and *L. maculicollis* ecology and lead to the development of alternative, ecologically sensitive and sustainable management tactics for the weevil.

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CURRICULUM VITA

BENJAMIN ALEXANDER M^{c}GRAW

EDUCATION:

2005 – 2009	Rutgers University, New Brunswick, NJ *Doctorate of Philosophy (Ph. D.)* Entomology
2002 - 2005	University of Massachusetts, Amherst, MA *Master's of Science*: Entomology
1993 - 1998	University of Maine, Orono, ME *Bachelor's of Science*: Animal Science

EMPLOYMENT:

2005 – 2008	Rutgers University, New Brunswick, NJ *Graduate assistant*
2002-2005	University of Massachusetts, Amherst, MA *Graduate assistant*
2001 - 2002	AgraQuest Inc., Davis, CA *Associate Biologist-Entomology*
2000 - 2001	Dow AgroSciences, Indianapolis, IN *Associate Biologist-Entomology/Biotechnology Division*
1998 - 2000	Mycogen Corporation/ Dow AgroSciences, San Diego, CA *Assistant Biologist-Entomology*

PUBLICATIONS:

McGraw, B.A. and A.M. Koppenhöfer. 2008. Population dynamics and interactions between endemic entomopathogenic nematodes and annual bluegrass weevil populations in golf course turfgrass. *Applied Soil Ecology*, In Press.

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