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INVESTIGATION INTO *LISTRONOTUS MACULICOLLIS* (COLEOPTERA: CURCULIONIDAE), A PEST OF HIGHLY MAINTAINED TURFGRASS

A Dissertation Presented

by

NIKKI LYNN ROTHWELL

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

DOCTOR OF PHILOSOPHY

May 2003

Department of Entomology

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INVESTIGATION INTO LISTRONOTUS MACULICOLLIS (COLEOPTERA: CURCULIONIDAE), A PEST OF HIGHLY MAINTAINED TURFGRASS

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by

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DEDICATION

This dissertation is dedicated to my gramma, Norma B. Rollet, for her unfaltering belief

that I could conquer the world if I set my mind to it.

ACKNOWLEDGMENTS

I would like to convey my gratitude to my advisor, Dr. Pat Vittum, for her continual support throughout my degree. Pat has taught me much about science, but more importantly, she showed me that there is more to life than just work. To my committee members, Drs. Anne Averill, Gail Schumann, and Beth Jakob for their guidance and dedication to my future. This university is lucky to have such a hardworking and devoted group of scientists working for it.

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In heartfelt appreciation to all my fellow graduate students: knowing we were all in 'it' together made some of the most unbearable days seem doable. Aaron Haselton, a

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ABSTRACT

INVESTIGATION INTO *LISTRONOTUS MACULICOLLIS* (COLEOPTERA: CURCULIONIDAE), A PEST OF HIGHLY MAINTAINTED TURFGRASS

MAY 2003

NIKKI ROTHWELL, B.S., WESTERN MICHIGAN UNIVERSITY M.S., MICHIGAN STATE UNIVERSITY Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST Directed by: Dr. Patricia Vittum

Listronotus maculicollis (Dietz) is a major pest of golf course turf in the northeastern United States. Because the larval stage causes considerable damage to short-mowed turfgrass, superintendents rely on chemicals for control. I investigated physiological, ecological, and behavioral characteristics of *L. maculicollis* to enhance management strategies that will lead to reduced insecticide inputs on golf courses.

Among turfgrass species, *L. maculicollis* larvae are reportedly found primarily in *Poa annua* L., annual bluegrass, among turfgrass species. To confirm this observation, I conducted an investigation to determine how abundant larvae were in *P. annua* compared with other grasses. *L. maculicollis* larvae were present in similar numbers in highly maintained grasses (*P. annua* and *Agrostis palustris* Huds., creeping bentgrass) in field studies, but in choice and no-choice tests, larvae were significantly abundant in *P. annua*.

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More *L. maculicollis* were collected in non-fertilized turf compared to fertilized turf, and more larvae were collected in short-mowed plots than from long-mowed plots.

I examined the distribution of adults and larvae across the width of a golf course fairway, but no differences were detected. Although no more larvae were detected at fairway edges, we determined by visual assessment that the turf on the edge of the fairway was poorer quality. I also established that adult *L. maculicollis* emerge from overwintering sites and walk onto host plants in the spring.

Because management strategies for *L. maculicollis* on turfgrass revolve principally around chemical applications, I refined a prediction system to enhance timing of control measures. I established 13.3° C as the base temperature for larval growth, and by using this temperature in a degree-day equation, I predict initial spring larval presence between 150 DD and 200 DD. Adult activity peaked at 20° C, and this was set as the temperature for golf course superintendents to target emerging spring adults to prevent subsequent larval infestation. Dissections suggest that May and August are the primary oviposition months and male and female reproductive organs increase in size during these months. Taken together, my studies improve our understanding of this pest's life cycle on golf course turf, and enhance effectiveness of management approaches.

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CHAPTER 1

REVIEW OF THE LITERATURE

The Biology of Listronotus maculicollis and other Closely Related Curculionidae

Listronotus maculicollis is the most damaging pest on golf course turf in the Northeast (Vittum 1999). The larval stage of this insect feeds on the short-mowed grass of tees, greens, and fairways causing significant damage. Because little information on *L. maculicollis* is documented, golf course superintendents apply multiple costly chemical applications (Cameron and Johnson 1971c, Vittum 1999) to prevent damage from this pest. In addition, few basic biological studies have been conducted on this insect, so the literature does not contain adequate information on *L. maculicollis*. Therefore, the purpose of this review is to apply associated literature of closely related weevil species to provide a historical background for our current research on *L. maculicollis* as a golf course pest.

Geographical Distribution of Listronotus maculicollis

Listronotus maculicollis (Dietz), often known as the annual bluegrass weevil, is a native pest of highly maintained turfgrass in the northeastern United States. *L. maculicollis* was first reported damaging golf course turf in Connecticut in 1931 (Schread 1970, Vittum et al. 1999). The insect was noticed damaging golf course turf in 1957 and

again in 1961 by the Nassau County Cooperative Extension Association of New York State (Cameron 1970). In 1965, *L. maculicollis* caused significant turfgrass damage along the shores of Long Island, NY. Two years later, weevils were found in Westchester County, NY, just north of New York City. In the same year, golf course superintendents detected *L. maculicollis* damage on their courses in Ithaca, NY (Vittum et al. 1999). Weevils damaging highly maintained grass were also collected from many golf courses in Connecticut as well as in the Pocono Mountain region of Pennsylvania (Britton 1932, Cameron et al. 1968, Cameron and Johnson 1971a, b).

Golf courses in the metropolitan New York and Long Island areas as well as southern Connecticut sustain the highest levels of damage from *L. maculicollis* (Vittum et al. 1999). However, damage has more recently been reported from many northeastern states including Massachusetts, Vermont, Rhode Island, New Jersey, southern New Hampshire, and isolated areas of Pennsylvania. In 2002, *L. maculicollis* was detected in Delaware and Maryland (S. Zonteck, personal communication). There have even been accounts of *L. maculicollis* damage to golf courses in Toronto and Montreal, Canada (J. Skorulski, personal communication).

Taxonomy of Listronotus maculicollis

Listronotus maculicollis belongs to the order Coleoptera, suborder Polyphaga, superfamily Curculionoidea, family Curculionidae, and subfamily Cylindrorhininae. There are 48 *Hyperodes* or *Listronotus* species listed in North America, north of Mexico (Kissinger 1964), and species within these particular genera often present complex classification challenges due to similarities in size and color. Henderson (1939) revised

the genus *Listronotus*, which resulted in reassigning five species previously in the genus *Listronotus* to the *Hyperodes* genus. According to Kissinger (1964), *Listronotus* is distinguished from *Hyperodes* by the second segment of the funiculus and the body length. In *Listronotus*, the second segment is much longer than the first while the weevil body is often more than 5mm. In *Hyperodes*, the second segment of the funiculus is slightly longer than the first segment, if not equal in length. Adult *Hyperodes* weevils are also somewhat smaller in size than closely related *Listronotus*--usually less than 4.5 mm. Based on this classification, Kissinger (1964) designated 28 species of weevils in the *Listronotus* genus while Henderson (1939) identified 33 species in this genus. Stockton (1956) agreed these genera were difficult to distinguish. Pottinger (1961a) summarized the difficulty in differentiating *Hyperodes* from other closely related genera, which includes *Listronotus*.

The scientific name *L. maculicollis* has undergone revision. This weevil was originally categorized as *Hyperodes* sp. near *anthracinus* (Warner 1965). Vittum (1979) placed this species between *anthracinus* and *maculicollis* based on its morphology, but eventually, Warner, an expert on the closely related, but distinct genera *Hyperodes* and *Listronotus*, reviewed both genera and designated the weevil under investigation to be *L. maculicollis* (Vittum et al. 1999). Additionally, Blatchley and Leng (1916), Stockton (1956), and Kissinger (1964) variously described the species of *Listronotus* (formerly *Hyperodes*) near *anthracinus* as having a glossy body surface with light pubescence and a prominent median prothoracic carina. *Listronotus* (formerly *Hyperodes*) *maculicollis*, on the other hand, has a faded exterior with thick pubescence and a reduced prothoracic carina.

Many common names have also been suggested for this insect. The most widespread common name for *L. maculicollis* is the annual bluegrass weevil. This name is derived from observational evidence that suggests *L. maculicollis* larvae prefer *Poa annua* L., annual bluegrass. However, given weevil feeding preference data presented here, this name may not be suitable to describe *L. maculicollis*. The name turfgrass weevil has also been proposed (Cameron 1970, Vittum 1979, Vittum 1980, Vittum et al. 1999).

General Weevil Information

Although taxonomic information on *L. maculicollis* exists (Vittum 1979), little biological knowledge of this weevil is currently available. Since most weevils are phytophagous (Arnett 1968) and survive on or in nearly all parts of plants (Dillon and Dillon 1961), we can begin with the Curculionid literature for a better understanding of weevil biology and apply that knowledge to *L. maculicollis*. According to Goldson (1979a), much of the Curculionid literature indicates that most weevils are comparable in both biology and morphology. We can further investigate more specific weevil literature, such as research concerning *Listronotus* spp. and apply it to our *L. maculicollis* studies. Beetles in the family Curculionidae are an exceptionally diverse group of organisms--over 60,000 species have been described (Anderson 2002). The majority of weevils feed on plant material (Arnett 1968, Arnett et al. 2002). Although some weevils are spow high

host specificity with their plant counterpart, and many groups of weevils have a narrow range of host plants (Arnett 1968, Arnett et al. 2002). Weevils in the genus *Listronotus* appear to be associated with a variety of plants (O'Brien 1981, Anderson 2002).

Listronotus spp. as Economic Pests

Three major pest species are found in the *Listronotus* genus: *L. oregonensis* (LeConte), *L. bonariensis* (Kuschel), and *L. maculicollis* (Dietz). *L. oreongensis*, known as the carrot weevil, was first reported on carrots in Illinois (Chandler 1926). This weevil also caused early damage to carrot crops in New Jersey (Pepper 1942) and in Massachusetts (Whitcomb 1965). The two other pest species, *L. bonariensis* and *L. maculicollis*, both cause severe feeding damage to plants in the family Poaceae. *L. bonariensis*, the Argentine stem weevil, was first discovered damaging wheat (*Triticum* spp.) crops in New Zealand in 1933 (Pottinger 1961a, b). This Curculionid pest feeds on wheat, barley (*Hordeum vulgare* L.), oats (*Avena* spp.), and pasture grasses, but appears to prefer short rotation ryegrasses (*Lolium* spp.) (Timlin 1964). *L. maculicollis* also feeds on grass plants in the family Poaceae, but this weevil demonstrates a noticeable preference for highly maintained turfgrass (Cameron 1970, Cameron and Johnson 1971a, b, Vittum 1979, 1980, Vittum et al. 1999).

Host Plant Use in Listronotus spp.

When an insect is presented with multiple food resources, it can feed on all resources in a particular range, or the insect can prefer one host over the others. Preference differences are often a result of insects using more than one host species (Singer 1983). Singer (1983) proposes two approaches to which insects come to feed on multiple hosts: 1) within a series of suitable hosts, insects may not have a preferred plant, so they feed on the first available host, or 2) all insects in a population prefer one host to to another, but some insects may not encounter their preferred host and are forced to feed on less favored plants. Since the relationship between insect host preference and the host itself is not explicit, we must first know the variation in host use before we can understand the differences in preference.

The evolution of host use centers around two major components: oviposition preference and larval performance (Gratton and Welter 1998). This relationship between oviposition behavior and larval survivorship is crucial in the evolution of host choice (Thompson 1988, Singer et al. 1994, Thompson and Singer 1998). Thompson and Pellmyr (1991) suggest oviposition behavior presents the primary origin for deviation of insect species onto differenct plant hosts. Oviposition choice by female insects appears to be the initial component in host use and ultimately host preference (Futuyma 1983). Oviposition is especially important when newly hatched offspring are relatively immobile, as with *L. maculicollis* larvae, and are unable to move a considerable distance to locate an appropriate host; thus, the ovipositing female makes the resource choice for her offspring (Sadeghi and Gilbert 2000). When a female insect is presented with different hosts in which to lay eggs, oviposition choice results in a hierarchy (Waldvogel

and Gould 1990, Nylin and Janz 1993, Mays and Kok 1997, Gratton and Welter 1998). The female lays the most eggs on the her most preferred plant, fewer eggs on her next preferred plant, and even fewer eggs on her least favored plant species. Therefore, female insects' oviposition choice may provide the initial evolutionary step in multiple host use among polyphagous herbivores.

Many insect species females will oviposit on non-host plants (Thompson 1988, Mayhew 1997). This phenomenon can take place: 1) when plants or insects are novel to a region where there has not been enough time for insects to evolve ideal associations between oviposition preference and larval survivorship (Chew 1977), 2) when adult female insects travel less than their larvae (Tammaru et al. 1995), or 3) when adult life span is extremely short and will not allow for selective host preference (Larsson and Ekbom 1995). Because golf course maintenance standards have increased dramatically in the past 20 years (Turgeon 1996), highly maintained golf course turf is a relatively new host for insects. Therefore, *L. maculicollis* may have just recently adapted to using these high input turfgrass species as hosts, but these plants may not be the optimal hosts for this insect. Additionally, new turfgrass cultivars are constantly bred and planted on golf courses, which may not allow these insects to evolve the ideal host plant-insect relationship necessary for optimal survival. Hence, female *L. maculicollis* may be forced to oviposit in less than suitable host plants.

Even if oviposition behavior provides the primary basis for multiple resource use, offspring fitness on the plant resource is thought to play a major role in host use (Thompson 1988, Berebaum 1990, Jaenike 1990, Futuyma and Keese 1992). Although host range of maturing larvae is often greater than their ovipositing adult counterparts

(Wiklund 1975, Courtney and Forsberg 1988), there is evidence of varied larval survivorship on differing hosts. Many insects have increased survivorship on plants preferred and eaten by their adult counterparts (Barker and Maczka 1996), but host plant ecological, physiological, and morphological characteristics determine the overall fitness of insect larvae. If plant species vary in their ability to provide adequate nutrients for growing larvae, larvae will show increased development on plants best suited to their needs. Hence, larvae use host plants that supply ample nourishment for optimal survivorship (Raupp and Denno 1983, Berenbaum and Zangerl 1992). Therefore, understanding both female oviposition selectivity and larval fitness across a variety of potential hosts is central to understanding diet breadth. By reviewing the literature of *Listronotus* pest species, we can relate the principles of feeding variation to *L. maculicollis*.

Although *L. oregonensis* is referred to as the carrot weevil, this insect has been known to feed on multiple umbelliferous plants: parsley (*Petroselinum crispum* (Mill.) Nyman), parsnips (*Pastinaca sativa* L.), and celery (*Apium graveolens* L.) as well as carrots (*Daucus carota* L.) (Chandler 1926, Boyce 1927, Pepper and Hagmann 1938, Pepper 1942, Whitcomb 1965, Grafius and Otto 1979, Simonet and Davenport 1981, Grafius and Collins 1986, Stevenson 1986). In Quebec and many areas of the northeastern United States as well as Illinois, *L. oregonensis* damages carrot more than the other umbelliferous crops (Chandler 1926, Boivin 1985, Grafius and Collins 1986, Stevenson 1986). Therefore, depending on its location, the carrot weevil demonstrates some type of variation in host plants, and in most cases, this insect primarily damages carrot. However, this weevil also causes noticeable damage in parsley and celery, and in some of the earlier literature, this insect is referred to as the parsley weevil (Chandler 1926, Boyce 1927).

Listronotus bonariensis feeds on a wide variety of plants, but more damage is seen on some species than others. Although this pest has been found damaging different grass species, such as oats, wheat, and barley, this weevil appears to prefer specific grasses (Kelsey 1958, Pottinger 1961a, b, Timlin 1964), especially those found in the lowland pastures of New Zealand. Short-rotation ryegrasses comprise the majority of pastures in these areas (Barker 1989b), and between the two most common species, *Lolium multiflorum* L.and *L. perenne* L., *L. bonariensis* has been shown to prefer *L. multiflorum* (Kelsey 1958, Pottinger 1961b, Goldson 1979b, Goldson 1982, Barker et al. 1984, Pottinger et al. 1985). However, *L. bonariensis* will attack perennial ryegrass, but tremendous variability in susceptibility exists between cultivars (Kain et al. 1977, 1982a, b, Gaynor and Hunt 1982, Dymock and Hunt 1987, Prestidge 1991). Barker et al. (1984), Barker (1989b), and Firth et al. (1993) have even shown that *L. bonariensis* feed on warm-season grasses, which are grown in pastures in Northern New Zealand (Lambert 1967, Rumball 1991).

In addition, *L. bonarensis* variability in host plants has been shown to be governed, in part, by cultural and environmental factors affecting New Zealand pasture grasses. Hunt and Mortimer (1981) found differences in *L. bonariensis* feeding on grass plants with different fertility (primarily nitrogen) regimes. Kain et al. (1977) observed significantly different levels of *L. bonariensis* feeding damage on two cultivars of *L. perenne* under well-fertilized, but moisture-stressed conditions. However, when these same two cultivars were placed under moisture-stressed surroundings with no fertilizer

application, there were no differences in *L. bonariensis* feeding damage (Kain et al. 1982b). Gaynor and Hunt (1981, 1982) reported differing levels of *L. perenne* susceptibility to *L. bonariensis* under variable nitrogen and moisture levels. Recent studies have suggested that other factors, such as presence or absence of endophytes in grasses, also play a role in grass susceptibility to *L. bonariensis* feeding. Therefore, from previous research, we conclude that *L. bonariensis* feeding is altered when pasture grasses receive differing fertilizer and water treatments.

Similar to *L. oregonensis* and *L. bonariensis*, *L. maculicollis* also exhibits variation in its host plants. These weevils feed most commonly in highly maintained, short-mowed turfgrass, such as golf course tees, greens, and fairways. These weevils are not regularly observed on home lawns (Vittum et al. 1999), where this grass is maintained at a higher cut than standard golf course tees, greens, and fairways (Turgeon 1996). Cameron and Johnson (1971b) have shown weevils to feed on clover (*Trofolium repens* L.), plantain (*Plantago* spp.), dandelion (*Taraxacum officinale* Weber), and mulberry (*Morus* spp.), but with no visible damage.

Golf course superintendents most often observe *L. maculicollis* damage in areas of golf courses with high infestations of annual bluegrass, *P. annua*. Additionally, *L. maculicollis* has been reported to primarily attack *P. annua*-infested portions of the golf course while leaving other grasses, such as creeping bentgrass (*Agrostis palustris* Huds.), untouched (Cameron and Johnson 1971a, b, Vittum 1980, and Vittum et al. 1999). Vittum and Tashiro (1987) reported that *L. maculicollis* feeding is limited to *P. annua*. Cameron and Johnson (1971c) and Tashiro (1976) suggested that what seems to be

feeding injury in creeping bentgrass might actually be *L. maculicollis* attacking undetected patches of *P. annua* among the creeping bentgrass plants.

In addition, Barker et al. (1989b) and Barker (1993) found higher Argentine stem weevil populations in *P. annua* and reported that ryegrass pastures with high *P. annua* encroachment had heavier infestations of *L. bonariensis*. These studies, in conjunction with implications of past *L. maculicollis* work (Cameron and Johnson 1971c, Tashiro 1976, Vittum and Tashiro 1987), suggest that *L. maculicollis* and *L. bonariensis* have an affinity for *P. annua*. However, results from our current studies suggest that *L. maculicollis* will feed on a variety of grass species.

Spring Emergence, Overwintering, and Distribution of Listronotus spp.

Ecological baseline information is necessary to manage insect pest species in cropping systems. By understanding insect behavior, we can provide commodity growers with strategies to control these pests. Overwintering and spring emergence patterns play an important role in predicting when and where insect pests infest crops. Means of movement, flying vs. walking, from overwintering sites in the spring influences control strategies for the first generation. After emergence, and once the insect has reached host resources, knowledge of its distribution within the cropping system will contribute to overall pest management. We will present information on the three pest species of *Listronotus* to provide a background of weevil behavior regarding overwintering, spring emergence, and distribution within their host plant systems. The pest weevils in the genus *Listronotus* have similar overwintering and spring emergence behaviors. Their methods of dispersal upon emergence from hibernation also are somewhat similar. These weevils overwinter in the adult stage, and as spring commences, they emerge from hibernation and begin to feed and oviposit.

The earlier literature provides conflicting reports as to the primary overwintering sites for L. oregonensis. Chandler (1926) observed carrot weevils overwintering in areas adjacent to the carrot crop, whereas Boyce (1927) suggested that L. oregonensis hibernates in the crop area. Boyce (1927) surmised that heavy manure applications to post-harvested carrot fields provided well-insulated overwintering sites for carrot weevils. However, most current studies suggest that carrot weevils hibernate a short distance away from food patches (Pepper and Hagmann 1938, Pepper 1942, Whitcomb 1965, Boivin 1985). To trap adult weevils as they emerged from overwintering sites, Grafius and Otto (1979) and Simonet (1981) used baits placed along field perimeters. This method of capture demonstrated that weevils could be captured as they entered host fields from outlying areas. Since carrots are not a perennial crop, L. oregonensis movements suggest that weevils to travel to and from host plants seasonally. Hence, carrot weevils must move from empty carrot fields to locate a suitable hibernation site. Grafius and Collins (1986) suggested that areas adjacent to crop fields are the spring weevil infestation sources.

The method of spring emergence of the carrot weevil also has been studied. Boyce (1927) never observed weevils in flight, whereas Pepper (1942) reported adults only fly on rare occasions. Pepper and Hagmann (1938) observed weevil flight, but they did not show weevils flying to host plants in the spring. When trapping methods were

employed, baited traps were placed on the ground, suggesting that weevils walk to the bait (Grafius and Otto 1979, Simonet 1981, Grafius and Collins 1986). Perron (1971) captured *L. oregonensis* in elevated light traps, but noticeably fewer weevils were caught by this method than with ground-level traps. Once weevils reach the crop area, there are few data to describe adult or larval *L. oregonensis* distribution on host plants.

Listronotus maculicollis has overwintering habits similar to those of *L*. oregonensis. *L. maculicollis* overwinters as adults in areas adjacent to their golf course host plants (Cameron and Johnson 1971b, Vittum 1980, Vittum et al. 1999). Cameron and Johnson (1971b) collected overwintering adult beetles in leaf litter and tufts of grass under trees bordering the golf course; however, no weevils were collected directly from their manicured host plant, fairways, tees, or greens. Vittum (1980) observed that weevil populations increased in white pine litter near infested golf courses in late August and September, and then decreased considerably in mid- to late April. These results suggest that adult weevils travel to locations adjacent to highly maintained golf course turf to hibernate and move back onto host plants in the spring. Since these short-mowed hosts serve as summer food sources, but cannot provide winter shelter due to their extremely short mowing height, *L. maculicollis* must move from host plants in the fall to a suitable overwintering locations.

Upon emerging from overwintering sites in the spring, Vittum (1980) suggested that *L. maculicollis* adults moved progressively through the rough-mowed turf to fairway perimeters, where primary oviposition occurs. Thus, overall summer distribution of adults and larval *L. maculicollis* would be on golf course fairway edges. Indeed, Vittum (1980) noted spring infestations were heaviest on fairways nearest overwintering sites.

Furthermore, golf course superintendents observe the majority of *L. maculicollis* damage on perimeters of their fairways, greens, and tees (N. Rothwell, personal observation).

The principal dispersal method of L. maculicollis in spring is suspected to be walking (Vittum 1980). This assumption is based on obvious turf damage along fairway, green, and tee perimeters, which are the areas that are first encountered by ovipositing females (Vittum 1980, Vittum and Tashiro 1987). However, weevils have been observed to disperse by flight as well (Cameron and Johnson 1971b, Vittum et al. 1999). Vittum (1980) caught adult weevils with a black light trap placed at a height of 1.5 m high, indicating that insects most likely flew to reach the elevated trap. Schread (1970) and Cameron and Johnson (1971b) also captured flying weevils on rectangles of plywood coated with yellow paint and sticky material. Cameron (1970) found more developed wing muscles in spring when L. maculicollis were emerging from hibernation, and in late June when second generation weevils were present; however, only 30% of insects had well-developed wing muscles. Vittum (1980) found a small percentage of weevils developed flight muscles in early June and mid-August. Regardless of the minimal flight muscle development, Vittum (1980) asserted that L. maculicollis adults walk onto host plants in spring.

Little information is available on the overwintering activities of *L. bonariensis*, but Whatman (1959) reported that weevils spend the winter in pastures. These pastures serve as *L. bonariensis* food and oviposition sources. Pottinger (1961a, b) found high weevil overwintering survival in the pasture grasses. Prestidge and van der Zijpp (1985) surmised that this increased survivorship is due to hibernation grasses doubling as favorable host plants. Because *L. bonariensis* does not move to and from overwintering

sites as *L. maculicollis* and *L. oregonensis* do, host plants of *L. bonariensis* play a duel role as both a food and a hibernation source. In addition, most regions of New Zealand experience milder winter conditions than those in the northeastern U.S. and Canada, where *L. maculicollis* and *L. oregonensis* are found, so *L. bonariensis* may not need to seek augmented protection as their *Listronotus* counterparts.

Although early flight observations of L. bonariensis were reported as mating flights (Kelsey 1958) and swarming flights (Morrison 1959), first detailed flight information of L. bonariensis was documented by Pottinger (1966). This study found frontal weather patterns to be the dominant factor directing L. bonariensis flight. Further studies corroborate that these insects do fly, but the causal factors remain under investigation. In fact, Prestidge and van der Zjipp (1985) reported that massed flights were absent altogether in particular regions of New Zealand. Because flight in L. bonariensis may be caused by a variety of factors, such as depleted food resources, overcrowded locales, reproductive status (Goldson 1981a, Prestidge and van der Zjipp 1985, Barker et al. 1989a, Goldson et al. 1999) or changing weather patterns (Prestidge 1966, Goldson et al. 1999), its flight activity appears distinctive from the occasional spring flights reported in L. maculicollis and L. oregonensis populations. The distribution of L. bonariensis in pasture grass has been difficult to estimate due to the insect's small size, different locations of each life stage within the pasture, and complex mobility and behavior of the adult weevil (Prestidge et al. 1985).
<u>Reproductive Seasonality, Oviposition Timing, and Population Dynamics of</u> <u>Listronotus spp.</u>

Management of *Listronotus* pests is based on the knowledge of their life histories. Basic information about insect pests, such as reproductive seasonality, timing of oviposition, and state of overwintering, is needed to provide a more extensive comprehension of insect life cycle and population dynamics. This knowledge, in turn, can provide growers with the tools they need to adequately manage these pests. Seasonality of other weevil species, especially cotton boll weevil (Anthomonus grandis Boheman) and alfalfa weevil (Hypera postica Gyllenhal) has been documented. Burke (1959) reported on the reproductive systems of the cotton boll weevil, while Brazzel and Newsom (1959) studied diapause and correlated seasonal development and reproductive systems to weevil activity in the fields. Many more authors have investigated boll weevil reproductive seasonality and its relationship to diapause and spring emergence (e.g. Earle and Newsom 1964, Lloyd et al. 1967, Walker 1967, Magnum et al. 1968, Tingle and Lloyd 1969, Walker and Bottrell 1970, Sterling and Adkisson 1974, Wade and Rummel 1978, White and Rummel 1978, Guerra et al. 1982, 1984, Carroll and Rummel 1985, Segers et al. 1987, Summy et al. 1988, Slosser and Fuchs 1991, Jones et al. 1992, Palmer and Cate 1992, Parajulee et al. 1996, Spurgeon and Raulston 1998). Similar studies have examined seasonal reproductive systems of the alfalfa weevil, Sitona cylindricollis Fahraeus, (Snow 1928, Campbell et al. 1961, Guerra and Bishop 1962, Tombes 1964, Pamanes and Pienkowski 1965, Bass 1967, Prokopy et al. 1967, Pitre 1969, Blickenstaff et al. 1972, Barney et al. 1978, Loan et al. 1983, Whitford and Quisenberry 1990, Elden

1995, Kuhar et al. 2000). Hans (1961) and Garth (1970) documented diapause in the alfalfa weevil. Of the three *Listronotus* species, the most extensive work has been done on *L. bonariensis* reproductive seasonality and diapause; these characteristics have led to better management strategies in pasture grasses. Reproductive diapause and seasonality have been documented in *L. oregonensis*, and preliminary information is available about *L. maculicollis* reproductive systems.

Oviposition has been investigated in *L. oregonensis*. Stevenson (1977) showed this insect to oviposit at variable times throughout the season, and Stevenson and Boivin (1990) suggest that this variability is attributed to seasonal variations in environmental factors. For instance, Simonet and Davenport (1981) reported that temperature influenced oviposition timing in Ohio. Moreover, increased air temperatures (between 20 and 30° C) augment carrot weevil egg production and decrease pre-oviposition time (Whitcomb 1965, Martel et al. 1976, Simonet and Davenport 1981). Boivin (1988) reported that carrot weevils required 147 degree-days (DD) (base temperature 7° C) in the field to initiate egg laying.

Photoperiod, another environmental factor that influences oviposition timing, is less understood in *L. oregonensis*. Whitcomb (1965) suspected that limited light provided the initial postponement in carrot weevil reproductive systems, and low temperatures further delayed the reproductive process. Ryser (1975) reported that females stop laying eggs in late August in the field in New Jersey, but when weevils were contained at 21° C and 16:8 light:dark (LD), they recommenced oviposition within two weeks. Stevenson (1977) found similar results. Stevenson and Boivin (1990) also showed that oviposition was initiated at high temperatures and long daylight hours;

however, field-collected females that had overwintered were not affected by photoperiod in the laboratory. Based on these studies, carrot weevil oviposition seems to be directed by a combination of photoperiod and temperature. Reduced ovarial development, as well as other reproductive organs, in response to environmental cues is called reproductive diapause (Borror et al. 1989).

Monitoring programs of the carrot weevil are based on these studies of reproductive seasonality. Boivin (1988) established a degree-day program to maximize insecticide use for carrot growers in Quebec. Monitoring programs have also been established for carrot weevil damage (Grafius et al. 1983, Boivin and Sauriol 1984, Stevenson 1985) based on monitoring techniques by Boivin (1985) and Stevenson (1985). Zhao (1991) created a simulation model to predict population dynamics of *L. oregonensis*.

Listronotus bonariensis also exhibits reproductive seasonality. Goldson (1979a) suggested *L. bonariensis* entered a reproductive diapause state induced by photoperiod. Ahmad (1978) alluded to *L. bonariensis* reproductive cessation in its native Argentina. Goldson (1981b) verified that *L. bonariensis* underwent reproductive diapause, and laboratory studies showed photoperiod triggered the onset of this state whereas sexual activity began again with increased hours of daylight. Barratt et al. (1995) found females subjected to LD 16:8 lay more eggs than weevils held in LD 12:12. Goldson and Emberson (1980) reported that *L. bonariensis* enter diapause in particular regions of New Zealand even when few temperature extremes are recorded; this reproductive cessation is similar to weevil diapause in Argentina. Weevils, which were recently introduced to New

Zealand are believed to continue to exhibit a relict diapause response as observed in their native South American habitat (Goldson and Emberson 1980).

Goldson (1979a, 1981b) documented that *L. bonariensis* have peak reproductive activity, as indicated by changes in female ovary size during summer months. Barker et al. (1988) chronicled the reproductive activity of *L. bonariensis* in northern New Zealand. Goldson and Emberson (1981) went on to provide detailed information of *L. bonariensis* functional reproductive morphology. Goldson (1979a) and Barker (1989a) described the reproductive organs of both male and female *L. bonariensis* used in these reproductive seasonality studies.

Using the information on reproductive seasonality and diapause, many model systems have been developed to manage *L. bonariensis* in pastures. In the laboratory, Barker (1988) established development times, in degree-days, for all stages and reported the following data: egg to larva, 75-91 DD (threshold temperature 10.1° C); larva to pupa, 159-246 DD (threshold tempeture 9.8° C); and pupa to adult, 149-204 DD (threshold temperature 10.4° C); and the total period for weevils to complete its life cycle took between 422-486 DD (threshold temperature 10.2° C). Previous studies estimating time of each *L. bonariensis* life stage were similar to those of Barker (1988) (Kelsey 1958, May 1961, Pottinger 1961a, Power and Singh 1974, and Goldson 1979a). Goldson et al. (1982) used computer modeling and determined the development threshold temperature of *L. bonariensis* to be 10° C. His larval degree-day accumulations were similar to the findings of Barker (1988), but egg and pupa degree-day units were slightly different. Ferguson et al. (1996) used the threshold temperature of 10° C to determine *L*.

bonariensis seasonal life cycle, and they established that weevil populations in the south of South Island, New Zealand had only one generation per year.

Knowledge of population dynamics has helped control L. bonariensis in the field. Barker and Pottinger (1982) conducted a preliminary study on the population biology of L. bonariensis, with regard to diapause and overwintering, in two locations in New Zealand. Barker et al. (1989b) found L. bonariensis populations maintained equilibrium as a result of density-dependent factors. They inferred: 1) weevil number is directly proportional to the number of the most beneficial host plants, 2) weevil populations will decrease as age of pasture increases, 3) re-seeding pastures with susceptible grasses will cause an upsurge in weevil population, 4) an insecticide application will only reduce grass damage in the generation of larvae to which it was applied, 5) insecticides sprayed for adults will not reduce larval numbers, and 6) biological control agents will only be effective if they can reduce weevil fecundity without reducing adult number. This study has proven to be helpful in controlling weevils in commercial pastures. However, accurately estimating weevil populations in pastures has proven to be difficult (Prestidge and van der Zijpp 1985), although many estimation techniques have been tried (Goldson 1978, Barker and Addison 1988, 1989, 1990).

Fewer studies have examined the reproductive seasonality or population dynamics of *L. maculicollis*, although the preliminary information of its life history is known. Adult weevils overwinter in leaf litter adjacent to golf course turf. In spring, they emerge and female *L. maculicollis* oviposit in the short-mowed grass. The eggs hatch, and first instars feed on the inner blades of grass (Cameron and Johnson 1971, Vittum and Tashiro 1987), and they travel down to the crown of the grass plant as they grow, where they feed

for the remainder of the larval stage. After completing five instars, larvae move down to the soil layer to pupate. Second-generation callow adults emerge a week later to repeat the cycle. Two to three generations have been reported in metropolitan New York area (Cameron 1970, Vittum 1980, Vittum and Tashiro 1987).

Because they found seasonal history uncertainty in *L. maculicollis*, Cameron (1970) and Cameron and Johnson (1971b) investigated *L. maculicollis* reproductive morphology. Cameron (1970) and Cameron and Johnson (1971a, b) reported that weevil activity throughout the season was complex, and that varying numbers of all life stages were found June through September. In addition, activity differed depending on sampling site. Vittum (1980) and Vittum and Tashiro (1987) investigated *L. maculicollis* seasonality and reported three significant conclusions: 1) population peaks were in mid-June and mid-July, 2) weevils have a varied number of generations per year, and 3) larvae undergo four molts in four to six weeks. No precise prediction model is currently used by turfgrass managers. The majority of *L. maculicollis* studies was initiated primarily to refine control strategies, and most are based around chemical applications (Cameron and Johnson 1971a, b, c, Tashiro and Straub 1973, Tashiro et al. 1977, Vittum 1980).

However, Vittum (1980) did develop a degree-day model for *L. maculicollis*, but it was only accurate in predicting summer-generation larvae. She found spring generation numbers were more directly correlated to calendar days than thermal units, and from these results, she suspected these weevils initiated spring activity based on photoperiod or a combination of photoperiod and temperature rather than temperature alone. Vittum (1980) provided preliminary threshold temperatures for a degree-day model. She tested the effect of temperature on larval molting at 15, 20, 25 and 30° C. At 12°C, larvae

survived but did not molt. At 15° C, larvae all died within 16-27 days of the experiment, and pupae molted into adults between 26-28 days. When larvae were held at 20° C, they molted in 8-9 days, and pupae molted in 8-10 days. At 25° C, molting time decreased to 4-6 days in larvae and 5-6 days in pupae. Finally, at 30°C, Vittum (1980) noted that both larvae and pupae molted in 4-5 days.

The most current research on *L. maculicollis* in golf course turf has investigated prospective natural enemies of this pest. Vittum and McNeill (1999) examined the potential of *Microctonus aethiopoides* Loan as a biological control agent, but with limited success; the wasp killed only 20% of *L. maculicollis* in laboratory conditions. Field trials conducted in 2001 and 2002 suggest there is potential for a *Steinernematid* nematode (*Steinernema* new sp.) and *Bacillus thuringiensis* var. *tenebrionis* to reduce larval populations in spring applications (P. Vittum, personal communication).

Purpose of Research

Because *L. maculicollis* is one of the most important economic pest of golf courses in the northeastern United States, the following chapters are an investigation into the biology and ecology of this insect on highly maintained turfgrass. We examined this insect's host preference by determining the primary location of *L. maculicollis* larvae among turfgrass species, and we also investigated the relationship between golf course maintenance practices and these larval populations. Our research established basic life history characteristics of this weevil, such as adult and larval distribution in golf course turf as well as the quality of the host plant and its association with this larval distribution. Adult spring emergence behavior was examined to recommend golf course management strategies for control of *L. maculicollis* damage. Another goal of our study was to build a precise forecasting system for golf course superintendents to accurately monitor *L. maculicollis* larvae and their associated damage. In addition, we studied adult temperature thresholds to provide superintendents with optimal recommendations for spring control treatments. Lastly, this research inspected the reproductive seasonality of *L. maculicollis* adults for a better understanding of the phenology of an economically important pest.

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CHAPTER 2

INVESTIGATION INTO THE ABUNDANCE OF *LISTRONOTUS MACULICOLLIS* IN DIFFERENT TURFGRASS SPECIES

<u>Abstract</u>

The effects of grass type, fertilization, and mowing height were investigated to determine the abundance of Listronotus maculicollis (Dietz) larvae on golf course turf. Previous research and superintendent observations have indicated L. maculicollis are more prevalent in Poa annua L. than other grass types. We found significantly more larvae in typically short-mowed grasses, *P. annua* and two creeping bentgrass, *Agrostis palustris* Huds., type 3way and cv. L93, than the long-mowed grasses, Kentucky bluegrass (P. pratensis L.) and Kentucky bluegrass/perennial ryegrass (Lolium perenne L.) mixture. However, in one study, L. maculicollis larvae from P. annua plots weighed more than those from the creeping bentgrass plots. We did not find differences among the shortmowed grasses in the field, but, in choice and no-choice studies conducted at the Turf Research Facility, significantly more larvae were detected in *P. annua* turf cores. We collected more larvae in non-fertilized turf than the fertilized plots, and mowing height played a role in larval location, but it could not be separated from grass species. L. maculicollis can reach damaging numbers in creeping bentgrass turf without the presence of P. annua.

Introduction

L. maculicollis, often known as the annual bluegrass weevil, is a pest of highly maintained turfgrass in the northeastern United States. Most damage is caused by the larvae of *L. maculicollis*. First and second instars feed within the grass stem, and older instars migrate outside the plant and progress down to the crown of the plant, where fifth instars cause the most severe damage (Tashiro and Straub 1973). If larvae are numerous enough, this damage is evident as large dead areas of turf on the golf course. Adults also feed on the grass, creating notches in the blades and weakening the grass, but because this feeding occurs well above the meristem, it causes no severe damage (Cameron and Johnson 1971a).

L. maculicollis larvae only damage close-cut, highly maintained turfgrass, as is found on golf courses (Vittum et al. 1999). Additionally and most importantly, observational data suggest that *L. maculicollis* larvae cause more damage to *P. annua* on golf courses than to adjacent creeping bentgrasses (Cameron 1970, Cameron and Johnson 1971a,b, Vittum 1980, Vittum and Tashiro 1987, Vittum et al. 1999). Cameron and Johnson (1971c) and Tashiro (1976) discovered *L. maculicollis* larvae feeding on what appeared to be creeping bentgrass, but these insects were actually feeding on previously unnoticed patches of *P. annua*. Vittum (1980) determined that *L. maculicollis* larvae feed solely on *P. annua* on golf course turf, but most of her work was performed in artificial laboratory conditions. *P. annua* in New Zealand is reported to be a recruitment grass for Argentine stem weevils, *L. bonariensis* Kuschel, in ryegrass pastures (Barker et al. 1989, Barker 1993). Furthermore, golf course superintendents repeatedly report that severe *L*.

maculicollis damage on golf courses is detected in areas with high infestations of *P*. *annua*. Superintendents also maintain that creeping bentgrass patches in the vicinity of *P*. *annua* patches are usually left undamaged by feeding *L*. *maculicollis* larvae, although there have been a few exceptions noted since 1995.

Although *L. maculicollis* has been observed to primarily damage *P. annua* on golf courses (Cameron 1970, Cameron and Johnson 1971a,b, Vittum 1980, Vittum and Tashiro 1987, Vittum et al. 1999), the abundance of *L. maculicollis* in different turf species has not been scientifically examined. The purpose of this research was to determine whether *L. maculicollis* larval populations are indeed more abundant in *P. annua* than other golf course grass species. Also, if *P. annua* was completely removed from test plots, we wanted to verify if *L. maculicollis* populations would reach damaging numbers. In addition, we also investigated the effects of two maintenance practices, fertilization and mowing height, on the abundance of larvae.

Methods and Materials

Testing the effect of grass type on abundance of *L. maculicollis* larvae. In 2000, plots of five grass types were constructed at Dunwoodie Golf Course, Yonkers, NY: greenhouse grown *P. annua*; *A. palustris*, cvs. 3way (33% Southshore, 33% L93, 33% Pennlinks) and L93; (Kentucky bluegrass, cvs. 20% Midnight, 20% America, 20% Liberator, 20% Award, and 20% Total Eclipse); and a mixture of *P. pratensis* and *L. perenne* (cvs. 20% Palmer III, 20% America, 20% Libertor, 20% Limousine, and 20% Total Eclipse). Five replicates of 0.61 m² x 0.46 m² plots were installed behind a golf course green, using a randomized complete block design contiguous to adult weevil overwintering sites. Previous observations indicated weevil damage at this location to be significant every year. Grass plots were mowed to optimal mowing heights: 3.81 cm for the Kentucky bluegrass and Kentucky bluegrass/perennial ryegrass mixture and 1.27 cm for the remaining grasses, which are standard for the turfgrass industry for fairway maintenance (Turgeon 1996). The plots received no herbicide, fungicide, or insecticide in any year of the study. A slow release fertilizer (1:2:1 NPK, 1.25 g N /m²) was applied on 15 April.

Plots were sampled for larvae on 2, 8, and 16 June 2000. Samples were collected using a 5.4 cm²-diameter turf-corer (Turf-Tec International[®], Oakland Park, FL). Five cores were removed on each of the dates, and were returned to the laboratory where they were hand-inspected for larvae.

Testing the effect of golf course stress and fertilizer. In 2001 and 2002, a split plot design of the same five grass types mentioned above was assembled in a low-stress area behind a green at Dunwoodie Golf Course. Five replicates of $0.61 \text{ m}^2 \times 0.46 \text{ m}^2$ plots were constructed in the same location used in the 2000 study. Low stress indicates golfers, golf carts, and maintenance equipment were minimally present on this location throughout the season. On 20 April, each plot was divided in half, and 2.44 g N/m² of fertilizer was applied to a randomly selected half. This treatment was repeated on 15 April 2002, but the rate of fertilizer was decreased to 1.25 g N/m² due to initial fertilizer burn the previous year.

In 2001, a duplicate set of plots of replicates was positioned on the east side of the fairway of the same hole, and were considered 'high-stress'. Fairway grass normally is subjected to more traffic, golfers, golf carts, and mowing equipment than grass located behind greens (Turgeon 1996). Fertilizer (2.44 g N/m²) was applied in the same manner as the low-stress plots in 2001.

We sampled for larvae 5 June 2001, and 3 June 2002, using the same turf-corer method mentioned above; ten plugs were taken from each half of each plot in 2001 and 2002. The turf cores were taken back to the laboratory in Amherst, MA where they were teased apart, and the larvae were counted and weighed individually on a Mettler PJ3600 balance. Larval sampling was repeated for second-generation *L. maculicollis* larvae on the high- and low-stress plots on 9 August 2001.

Testing the effect of mowing height. To test the effect of mowing height, plots using the same five turf species described above were installed behind green #11 at Dunwoodie Golf Course in 2001 and 2002. These plots were also positioned near known *L. maculicollis* overwintering sites. No herbicide, fungicide, insecticide, or fertilizer was applied to the plots throughout the duration of the study. The 0.61 m² x 0.46 m² plots were arranged in a split plot design where half of the plot was mowed to 1.27 cm, and the remaining half was maintained at 3.81 cm. This design was sustained for all grass types, regardless of optimal mowing height. Plots were "mowed" with electric hand-held clippers three times per week throughout the study period. Clippings were not removed after mowing.

Larval samples were taken with a 5.4 cm²-diameter turf-corer on two different dates each year: 5 and 12 June 2001 and 3 and 11 June 2002. Ten plugs were removed from each plot on each sampling dates for both years. Larvae were counted and weighed as above. Second-generation larvae were sampled similarly on 9 August 2001.

Turf Quality Ratings. In order to determine the impact of *L. maculicollis* larval feeding on the turfgrass, we performed quality ratings on all plots in 2002. Turfgrass quality ratings were determined weekly from 1 May to 3 June on fertilizer and mowing plots. We applied the standard qualitative turf measurement scale (National Turfgrass Evaluation Program (NTEP)) commonly used in turfgrass research (S. Ebdon, personal communication). We used the NTEP scale of 1 to 9 scale where 1 = brown, dead turf-unacceptable turf quality and 9 = dark green, lush, uniform turfgrass. A quality value of 6 is considered acceptable turf; any lower ratings indicate undesirable quality and higher values signify above-quality turfgrass. The quality of the turfgrass rating system combines the characteristics of color, density, and uniformity to provide a value to overall appearance of the turfgrass (Turgeon 1996). To minimize variability, turf quality was assessed between 0900 and 1000 each time.

Choice and no-choice tests. To test *L. maculicollis* abundance in different grass types in a controlled setting, we constructed choice and no-choice tests in 2002 at the University of Massachusetts Turfgrass Research Facility, South Deerfield, MA (Figure 2.1). Choice tests were designed to determine if given multiple host plants, *L. maculicollis* larvae would be more abundant in one grass host than another. No-choice tests were created to

determine whether larvae would be present in a given grass type, if no other option was provided. In other words, if offered no choice, we wanted to determine if the insects would survive on the provided grass type. Choice tests were performed in 52 cm x 38 cm x 28 cm Rubbermaid[©] storage units equipped with drainage holes. A 3:1 mixture of potting soil and sand covered the bottom one-third of each box. One standard turf-corer plug (16.1 cm²-diameter) of each of the five grass types, as described previously, was placed in a 32 cm-diameter concentric ring in the center of each box. Each box contained one core of each grass type (five total cores in each box) and the center of the box was devoid of turf. Plugs were buried into the mixture until the roots were covered. Five replications (including one set exposed to weevils and a "control" from which weevils were excluded) were placed on the turf farm grounds. In five boxes, 60 adult weevils, taken from overwintering sites, were placed directly in the center of the box. The remaining five boxes had no weevils and were used as a control to determine if poor quality turf resulted from environmental factors rather than larval feeding. All boxes were covered with a white mesh fabric sealed with duct tape to prevent weevil escape. Containers were watered daily, and the grass plugs were hand-trimmed to 2.0 cm weekly. The fabric was resealed after each grass cutting. Grass quality ratings, using the NTEP system, were taken weekly on all turf cores, from 6 May until the termination of the experiment on 15 June. On 15 June, plugs were removed from the containers and taken to the laboratory, where L. maculicollis larvae were counted.

No-choice tests were also set up at the research facility in South Deerfield, MA. One 16.1 cm² core of one of the five grass types was placed in a 33 cm x 33 cm x 16 cm wooden box containing 66% potting soil and 33% sand mixture. One third of each box

was filled with the soil/sand mixture, and each grass type was planted in the soil medium. Five replicates of each grass type were randomly arranged in an open field at the Turf Facility. Twenty-five adult *L. maculicollis* were placed in each box, and the boxes were sealed with mesh and tape, as described above. Boxes were irrigated each day as needed, and turf plugs were trimmed to 2.0 cm weekly. Turf quality was assessed each week for six weeks. On 15 June, turf plugs were removed from the field and hand-examined for larvae.

Creeping bentgrass trials. In 2000, we designed a preliminary test to determine if we would find more *L. maculicollis* in *P. annua* or adjacent creeping bentgrass turf on an established golf course fairway at Minnesceongo Golf Club in Pomona, NY on 12 June 2000. Sixty turf cores were removed from a fairway with visible *L. maculicollis* damage with a 5.4 cm²-diameter turf-corer; 30 cores were collected from areas with nearly 100% creeping bentgrass cv. Southshore, while the remaining 30 samples were gathered from areas containing a minimum of 50% *P. annua*. Because *P. annua* produces seed heads throughout the growing season, we were able to identify locations with high concentrations of this grass with a high degree of confidence. All cores were returned to Amherst and examined for weevil larvae.

Based on results from 2000, we designed an experiment to determine whether *L*. *maculicollis* larvae were more prevalent in different varieties of creeping bentgrass excluding *P. annua* in 2001 and 2002. Plots of five creeping bentgrass cultivars were installed on a golf course fairway at Minnesceongo Golf Club on 1 May 2001 and 20

April 2002. Creeping bentgrass cultivars were selected based on common golf course turf usage in the northeastern United States (S. Ebdon, personal communication): 3way (33% Southshore, 33% L93, 33% Pennlinks), L93, Penncross, Penntrio (33% Penncross, 33% Pennlinks, 33% Eagle), and Southshore. Five replicates of $0.91 \text{ m}^2 \times 0.46 \text{ m}^2$ plots were arranged in a complete block design on the edge of a fairway, bordering the rough. The fairway was chosen for plot installation based on previous observation of *L. maculicollis* damage. All culitvars were mowed to 1.11 cm throughout the trial, and no insecticides or fungicides were applied to the plots during the study. On 7, 14, and 21 June 2001, we collected ten cores (5.4 cm²-diameter), transported them to Amherst, and examined each core for larvae. In 2002, we collected 20 turf cores from each plot on 6 June. All cores were returned to the laboratory, where larvae were counted.

Nutrient Analysis of Grasses. To determine if host plant nutrients had an effect on abundance of *L. maculicollis* larvae, grass from all plots from Dunwoodie Golf Club (fertilizer and mowing height plots) was analyzed for nutrient content in 2000 and 2001. We determined the percent of nitrogen, potassium, and phosphorus using NIRsystems scanning spectrophotometers (Near Infrared), mode 591, Karsten Turf, Inc., Phoenix, AZ. Leaves and stems from each plot were cut in the field with hand-held clippers. Grass was brought back to the laboratory and dried in an oven at 93° C for two days. The dried grass was ground to a fine powder and analyzed by the spectrophotometer. Plots were not analyzed in 2002 because after larval samples were removed, there was not enough intact grass remaining in each plot to obtain representative samples. Statistical Analysis. Mean number of *L. maculicollis* larvae in the fertilizer study was analyzed for treatment effects by a parametric three-way analysis of variance (ANOVA) with year, fertilizer, and grass type as factors; the interactions between the factors were also analyzed. We also used this analysis on the mowing height study with year, mowing height and grass types as factors. Interaction terms were also analyzed with this test. Creeping bentgrass trials were analyzed with a two-way ANOVA using the factors of year and grass type. The interactions between these factors were also analyzed. Grass type and weevil/no-weevil were the factors tested in the choice and no-choice tests with a one-way ANOVA. Turf quality ratings were analyzed using a two-way ANOVA test with grass factor and fertilization or mowing height as the other factor. Nutrient analysis was analyzed with ANOVA. Differences among all these treatment means were tested using Tukey's Pairwise Comparison at $\alpha = 0.05$. Single regression analysis was used to determine the relationship of turfgrass qualities over time. Statistical analyses of field data were performed using Minitab[®] (2000) software.

Results

Testing the effect of grass type on abundance of *L. maculicollis* **larvae.** Results from this study can be seen in Figure 2.2. Based on our analysis, we detected significant differences in the abundance of larvae between the grass types (F $_{4, 99} = 4.85$; P = 0.001). Based on a Tukey's Pairwise Comparison, no significant differences were detected between the short-mowed grass plots, *P. annua*, and creeping bentgrass types L93 and

3way (P > 0.05), but we did see differences between the short-mowed plots and the longmowed plots (P < 0.05).

Grass analysis data for 2000 are illustrated in Figure 2.3. The mean percent nitrogen was significantly different in *P. annua* plots than in the remaining grasses (F $_{4, 23}$ = 13.9, P < 0.001). No differences were detected in percentage of potassium and phosphorus.

Testing the effect of golf course stress and fertilizer. The ANOVA comparing the affects of fertilizer and grass type on abundance of *L. maculicollis* larvae was significant (Table 2.1). In both years, we collected very few larvae from the long-mowed grasses (Kentucky bluegrass and Kentucky bluegrass/perennial ryegrass mixture). When we compared means between the short-mowed grasses, we found no significant differences (Tukey's Pairwise Comparison, P< 0.05). In 2001, we collected (mean \pm SE) 4.1 \pm 0.849, 3.2 \pm 1.16, and 1.5 \pm 0.411 larvae from *P. annua*, creeping bentgrass types 3way and L93, respectively (P < 0.05). Mean numbers of larvae collected in 2002 were similar (mean \pm SE): 3.7 \pm 0.97, 3.1 \pm 0.98, and 4.7 \pm 01.5. The high-stress grass plots of 2001, in the fairway, had too few larvae for analysis. Therefore, we could not assess stress as a factor of *L. maculicollis* larval location. Additionally, second-generation samples collected in August 2001 did not have enough larvae for analysis.

Fertilizer, as well as grass type, had an effect on the abundance of *L. maculicollis* larvae (F $_{1,99} = 5.26$; P = 0.024, Figure 2.4). In 2001, non-fertilized plots contained 68.0 \pm 0.942 larvae whereas 31.0 \pm 0.357 larvae were collected from fertilized plots. Data from 2002 show similar results.

Larval weights were significantly different between grass types (F $_{2, 83} = 12.96$; P < 0.001, Figure 2.5) in the low stress, fertilizer study. In 2001, heaviest larvae were collected from *P. annua* plots, where they weighed an average of 4.8 mg ± 0.695, which, according to Tukey's Pairwise Comparison, was significantly heavier than larvae collected from creeping bentgrass types 3way and L93. When we compared means in 2002, larvae from *P. annua* plots were again heavier than larvae from the creeping bentgrass types in 2002 (Tukey's Pairwise Comparison, P < 0.05). Too few larvae were found in the Kentucky bluegrass and Kentucky bluegrass/perennial ryegrass plots for analysis.

When grasses were analyzed for nutrient availability in 2001, we found no differences in nitrogen, phosphorus, or potassium (Figure 2.6), which contradicts the nutrient analysis from 2000.

When we examined the quality of turf in the low-stress, fertilized plots in 2002, we found no differences between fertilized and non-fertilized plots of the same grass type (Table 2.2). In addition, grass quality did not decline over the duration of the study (Table 2.3). Turf quality varied among the five grass types over the five weeks of the study, and in the final week of the study, Kentucky bluegrass/perennial ryegrass mixture plots had significantly better quality than the short-mowed turfgrasses and the Kentucky bluegrass plots (F $_{4,49} = 16.42$; P = 0.001).

Testing the effect of mowing height. Data from the mowing height trials of 2001 and 2002 (Table 2.4) were similar to those reported from the fertilizer study. Grass type significantly influenced the abundance of *L. maculicollis* larvae (F _{24, 99} = 3.30; P =

0.0002, Figure 2.7). The block effect was also significant (Table 2.4). When mean numbers of larvae were compared between grass types, significantly more larvae were collected from *P. annua*, and creeping bentgrass types 3way and L93 than Kentucky bluegrass and Kentucky bluegrass/perennial ryegrass mixture (Tukey's Pairwise Comparison, P < 0.05) and no differences in larval number were detected among the three short-mowed grass types (Tukey's Pairwise Comparison, P > 0.05). Mowing height also influenced the abundance of larvae among the five grass types as they were more commonly collected in short-mowed plots than long-mowed plots (F _{5,99} = 2.46; P = 0.045). In 2001, all grass plots mowed to a short height contained more larvae than their long-mowed counterparts. However, in the following year, more larvae were found in long-mowed creeping bentgrass cv. L93 than short-mowed cv. L93, and similar results were found in Kentucky bluegrass and Kentucky bluegrass/perennial ryegrass plots, but only four larvae were recovered from these plots during the two-year study. No larvae were recovered from any of the plots in the August sampling in 2001.

Weights of larvae recovered in the mowing height study varied with year. In 2001, no differences in larval weights were observed between the three short-mowed grasses (F $_{2,39} = 2.11$; P = 0.136, Figure 2.8). However, in 2002, larvae collected from *P. annua* and creeping bentgrass type 3way weighed significantly more than larvae found in creeping bentgrass cv. L93 when we compared means with Tukey's Pairwise Comparison (P < 0.05). Larvae collected from short-mowed *P. annua*, and the two creeping bentgrass types weighed significantly more than larvae collected from short-mowed *P. annua*, and the two creeping bentgrass types weighed significantly more than larvae collected from their long-mowed counterparts (F $_{1,48} = 10.9$; P = 0.002)

Grass type affected the overall analyis of nitrogen level (F $_{4, 49} = 4.06$; P = 0.007, Figure 2.9), but when we compared the means of nitrogen level, only *P. annua* and Kentucky bluegrass were significantly different (P < 0.05). No differences in phosphorus (P) (F $_{4, 49} = 2.30$; P = 0.75) or potassium (K) (F $_{4, 49} = 1.75$; P = 0.158) were detected among the five grass types.

Turf ratings for the 2002 mowing study varied throughout the five weeks of the study (Table 2.5). In the first and fourth weeks of the study, short-mowed turf plots were rated significantly lower in quality than the long-mowed plots (F $_{4,49}$ = 3.10; P = 0.026 and F $_{4,49}$ = 11.38; P = 0.002, respectively). Week two and five showed a trend of short-mowed plots with lower quality than their long-mowed counterparts (F $_{4,49}$ = 2.39; P = 0.067 and F $_{4,49}$ = 11.38; P = 0.002, respectively). There was no significant decline in turf quality in any grass type throughout the study (Table 2.6).

Choice and no-choice tests. When larvae were collected from the grass cores in the choice test, differences between the grass types was obvious, unlike field tests at the golf course. Grass type determined the abundance of larvae (F _{4, 24} = 17.54; P < 0.001, Figure 2.10), and we collected more larvae from *P. annua* plots (Tukey's Pairwise Comparison, P < 0.05) than any of the other grass types.

Turf quality ratings can be see in Table 2.7. Little change in grass quality was noted until the final week of the study. When we compared the quality of grasses in week six, we found significant differences among the grass types (F $_{1,49} = 16.11$; P < 0.001), and *P. annua* had the lowest turf quality (Tukey's Pairwise Comparison, P < 0.05). According previous knowledge of larval growth, we surmised *L. maculicollis* larvae

would be entering fourth and fifth instars (penultimate and ultimate stages) during the fifth week of the study when *P. annua* was observed to have unacceptably low quality. When quality of individual grasses was compared in the choice test over the six weeks of the study, no grass type showed a significant decline in quality (Table 2.8) from week one to week six.

Results from the no-choice tests at the Turf Research Facility were similar to the choice test (F $_{4, 24} = 16.77$; P < 0.001, Figure 2.10). When the ANOVA was followed by Tukey's Pairwise Comparison, *P. annua* cores had significantly more larvae compared with the other grasses (P < 0.05).

Turf quality patterns in the no-choice tests were also comparable to those of the choice tests (Table 2.9). The first three weeks of the study revealed no differences in the qualities of the five turfgrasses. However, in the fourth week, *P. annua* plugs were significantly lower in quality than creeping bentgrass type 3way, Kentucky bluegrass, and Kentucky bluegrass/perennial ryegrass mix (F $_{4,24} = 7.16$; P = 0.001). *P. annua* had lower quality than the other grass types in weeks five (F $_{4,24} = 33.67$; P < 0.001) and six (F $_{4,24} = 27.83$; P < 0.001). *P. annua* was the only grass to decline in quality.

Poa annua demonstrated the most dramatic decline in turf quality throughout the duration of the no-choice study (Table 2.10). In weeks 1-3, *P. annua* did not deteriorate in quality compared to the other grass types, but *P. annua* had an unacceptable quality rating of 2.0 ± 0.592 in the final week of the experiment (F _{1, 29} = 52.16; r² = 0.651; P < 0.001).
Creeping bentgrass trials. During the preliminary investigation of 2000, we performed on a damaged fairway, more larvae were found in *P. annua* plugs than in the creeping bentgrass cv. Southshore cores. We found 78 ± 3.2 larvae in the *P. annua* cores compared with 8 ± 2.3 larvae in the creeping bentgrass cores.

In 2001, we found significant differences between the five cultivars of creeping bentgrass (F 4, 74 = 4.34; P = 0.003, Figure 2.11). When we compared mean numbers of larvae, we found creeping bentgrass type 3way had significantly more larvae than Penncross, Penntrio, and Southshore (Tukey's Pairwise Comparison, P < 0.05). Cultivar L93 plots recovered 27 \pm 0.794 larvae, but these numbers were not different from creeping bentgrass type 3way (Tukey's Pairwise Comparison, P > 0.05).

When we repeated this experiment the following year, we found similar numbers of *L. maculicollis* larvae in the creeping bentgrass cultivars (F _{4, 24} = 4.93; P = 0.006). Creeping bentgrass types 3way and L93 contained the highest number of *L. maculicollis* larvae (Figure 2.11), but when we compared the means, significant differences were only detected between the cultivars of L93 and Penntrio and between the cultivars of L93 and Southshore (Tukey's Pairwise Comparison, P < 0.05).

This study shows a significant fluctuation in number of larvae from one year to the next (F $_{1,149} = 27.76$; P < 0.001). The differences in numbers of larvae collected from grass types also varied with year (F $_{1,149} = 4,57$; P = 0.002). Creeping bentgrass cv. L93 plots yielded (mean ± SE) 1.8 ± 0.751 larvae in 2001 and 7.5 ± 1.82 in 2002. Type 3way also showed increases in numbers from 2001 to 2002.

Discussion

Contrary to observational data, our field results did not show L. maculicollis larvae to be more abundant in *P. annua* than other golf course grasses. As noted previously, golf course superintendents report more L. maculicollis damage to areas of their golf courses that have high infestations of *P. annua*, and many studies that have shown this weevil species to damage primarily P. annua turf (Cameron and Johnson 1971a, Tashiro 1976, Vittum 1980, Vittum et al. 1999). However, after three years of experimentation, L. maculicollis larvae were uniformly located in the grass species commonly mowed to fairway height: two types of creeping bentgrass, 3way and L93, and *P. annua*. We did not find significantly higher numbers of larvae in the *P. annua* plots than the creeping bentgrass types, whereas oviposition and/or larval survivorship was lower in Kentucky bluegrass and the Kentucky bluegrass/perennial ryegrass mixture. In contrast to these field tests, in both choice and no-choice tests conducted at the Turf Research Facility, the highest numbers of larvae were collected from P. annua. P. annua plays a role in the abundance of L. bonariensis in New Zealand pastures. Barker et al. (1989) and Barker (1993) concluded pastures with infestations of P. annua resulted in higher populations of L. bonariensis than pastures without P. annua, but we could not show L. maculicollis larvae to be more abundant in P. annua plots in our field studies.

To ultimately understand insect host preference, we must first investigate the variation in host use. Our field results demonstrate *L. maculicollis* development varies on different grass species. The use of multiple hosts has also been shown in other species of

Listronotus. L. oregonensis (LeConte) has been shown to feed on celery, parsnips, parseley, and carrots (Chandler 1926, Boyce 1927, Pepper and Hagmann 1938, Pepper 1942, Whitcomb 1965, Grafius and Otto 1979, Simonet and Davenport 1981, Grafius and Collins 1986, Stevenson 1986). In Quebec and many areas of the northeastern United States as well as Illinois, L. oregonensis damages carrot more than the other umbelliferous crops (Chandler 1926, Boivin 1985, Grafius and Collins 1986, Stevenson 1986). However, this weevil also causes noticeable damage in parsley and celery, and in some of the earlier literature, this insect is referred to as the parsley weevil (Chandler 1926, Boyce 1927). Ultimately, L. oregonensis host use appears variable depending on geographic location. L. bonariensis also displays variation in host use. Although this pest has been found damaging different grass species, such as wheat (Triticum spp.), barley (Hordeum vulgare L.), oats (Avena spp.), this weevil appears to prefer grasses found in the lowland pastures of New Zealand (Kelsey 1958, Pottinger 1961a, b, Timlin 1964). Short-rotation ryegrasses comprise the majority of pastures in these areas (Barker 1989), and between the two most common species, Lolium multiflorum L.and L. perenne L., L. bonariensis has been shown to prefer L. multiflorum (Kelsey 1958, Pottinger 1961b, Goldson 1979, Goldson 1982, Barker et al. 1984, Pottinger et al. 1985). However, L. bonariensis will attack perennial ryegrass, but tremendous variability in susceptibility exists between cultivars (Kain et al. 1977, 1982a, b, Gaynor and Hunt 1982, Dymock and Hunt 1987, Prestidge 1991). We know that if plants vary in their ability to provide adequate nutrients for growing larvae, larvae will survive on the plants best suited to their needs. From our field studies, we observed varying abundance of larvae in different turfgrass species, which suggests L. maculciollis can survive on multiple grass species.

The creeping bentgrass trials provide additional evidence that *L. maculicollis* larvae can feed on different host grasses other than *P. annua*, as no *P. annua* was present in the test plots.

Although L. maculicollis appear to use multiple hosts, weight, which is often a measure of larval fitness, was higher in insects collected from P. annua in our fertilizer trial. However, since we weighed all larvae recovered but no distinction was made as to the instar of each larva, we do not know if larvae in *P. annua* plots were later instars, which would account for increased weight. On the other hand, larvae collected from all grass types, including P. annua, could have been in the same instar, but insects collected from *P. annua* plots weighed more because this grass may have provided supplemental nutrients for growing larvae. As plant-herbivore interactions are often quantified by measuring the consumption and usage of host plants by phytophagous insects (Scriber and Slansky 1981, Slansky and Scriber 1985, Tabashnick and Slansky 1987), when herbivorous insects are supplied with a choice of hosts, behavioral or physiological responses to these host choices can reveal nutritional differences in larvae. For example, if an insect has a lower growth rate when fed one host plant than another, the insect may consume the plant at a slower rate, which will ultimately reach ultimate weight, but require more feeding time. Additionally, the insect may not convert the resource to biomass efficiently, and either of these responses would result in low-weight herbivores (Meade and Hare 1991). On the other hand, if herbivore growth rates do not differ between host resources, then we can assume there are no differences among host plants or insects compensate for deficient hosts with excessive consumption (Meade and Hare 1991). Therefore, larval weight can be an initial measure of the nutritional effects a host

plant has on its herbivore, and in our case, larval weight was higher in *P. annua* in one trial.

The influence of fertilizer on insect biology and physiology has been studied for many insects (McNeill and Southwood 1978, Tingey and Singh 1980, Dowell and Steinberg 1990), but results vary with insect and plant species. The majority of studies illustrate that insects feed, grow, and damage fertilized plants more readily than plants with low fertilizer levels, but 25% of those trials show a negative correlation or are inconclusive (Scriber 1984). In our study, fertilizer had a limiting effect on *L. maculicollis*. We collected more larvae from non-fertilized plots than the fertilized plots; however, based on this study, we cannot determine whether the fertilizer was a deterrent to adult females or whether it adversely affected *L. maculicollis* larvae. The fertilizer treatment was applied at the time of *L. maculicollis* oviposition, and we suspect it discouraged females from laying eggs in the fertilized turf. The sweet potato weevil, *Cylas formicarius elegantulus* (Summers), laid fewer eggs in fertilized sweet potatoes than non-fertilized plants (Mao et al. 2001).

Our results conflict with studies performed on *L. bonariensis* where nitrogen applications have amplified damage in ryegrasses (Gaynor and Hunt 1982, Hunt and Gaynor 1982, Hunt et al. 1988). However, these fertilizer applications were made when larvae were present in the plots, not just at peak oviposition time. In addition, grass cultivars affected the placement of *L. bonariensis* larvae more than the influence of fertilizer. Furthermore, our initial data from 2000 showed less nitrogen in plant tissue of *P. annua*, but no more larvae were found in those plots than either creeping bentgrass type L93 or 3way. Prestidge et al. (1985) found no differences in growth of New Zealand

grass grubs, *Costelytra zealandica* (White), with increased nitrogen, although grass root growth increased with the fertilizer.

Mowing height of golf course turf has been discovered to play a major role in the location of turfgrass pests (Gyrisco et al. 1954, Potter et al. 1996, Smitley et al. 1998, Rothwell and Smitley 1999). Rothwell and Smitley (1999) demonstrated that turf height alone determines the location of adult and larval *Ataenius spretulus* Haldeman populations. *A. spretulus* populations were almost three-fold more abundant in short-mowed fairway turf than the long-mowed rough. *Cyclocephala* spp. were less abundant and weighed less in long-mowed plots than in short-mowed counterparts (Potter et al. 1996).

The height of the grass host of *L. maculicollis* influenced the abundance of larvae in our studies as we collected significantly more larvae in the short-mowed turfgrass. These results may have implications for turfgrass managers since previous research has shown many turfgrass pests to be preferentially located in short-mowed turf, including *A. spretulus, Aphodius granarius* L., *Cyclocephala* spp., and now *L. maculicollis*. Potentially, with more research, we can recommend a longer height of cut in highly maintained areas to minimize turfgrass pests. Additionally, since we noted short-mowed turf to be of lower quality than long-mowed turfgrass in many of our trials, these results indicate that short-mowed turf, regardless of type, is able to withstand less turfgrass pest feeding.

The act of mowing the plant itself may also influence turfgrass insects. Mowing grass may increase allocation of energy to the leaves at the expense of roots, crowns, and flowers (Reuss et al. 1983), as *L. maculicollis* adults notch the leaves of grass plants

(Cameron and Johnson 1971a) and oviposit there. Therefore, repeated mowing of shortmowed turfgrasses may draw adult females to feed initially on these grasses in the spring and ultimately oviposit there, which would result in higher numbers of subsequent larvae in grasses that are mowed on a frequent basis.

Since we found no differences in abundance of L. maculicollis larvae in highly maintained grass types, we investigated the ability of the five grasses to withstand weevil feeding. Potentially, superintendent observations of increased L. maculicollis damage to P. annua may be related to this grass's inability to survive large numbers of feeding larvae rather than more insects feeding on this grass species. The perennial form of this grass is considered a weed in closely mowed areas (Tutin 1957) because it displays intolerance to extremes such as temperature and moisture (Gibeault 1966, Beard 1982, Mitch 1998). P. annua also develops a shallow root system (Sprague and Burton 1937) under compacted conditions. Compaction in conjunction with the aforementioned extremes may again result in highly visible injury. Therefore, during stressful summer months, pressure from feeding larval populations may be an additional strain on already stressed grass. All of these factors may contribute to obviously discernible damage to P. annua on golf course turf, especially when this grass is compared to adjacent, more resilient turfgrass species. Based on our visual assessment, we did not observe increased damage in P. annua turf in the preference fertilizer and mowing experiments, but we detected diminished turf quality in P. annua cores at the Turfgrass Research Facility trial. However, our grass rating system was only implemented in 2002, so a more comprehensive examination needs to be done to verify how P. annua withstands L. maculicollis larval feeding.

From this research, mowing height, fertilization, and grass species all appear to influence the abundance of *L. maculicollis* larvae, but none of these factors prevail as a single factor that determines where *L. maculicollis* larvae feed on golf course turf. Our research suggests that future investigations should measure the impact of irrigation and compaction on golf course grasses in relation to *L. maculicollis* larvae. Investigation of individual grass species and their abilities to withstand larval feeding needs to be conducted in order to explain the repeated observations of increased damage as a result of *P. annua* on golf courses. In these studies, we only tested one biotype of *P. annua*, and since many more exist, more testing should be conducted to verify if weevil larvae prefer different biotypes of *P. annua*. This grass species also has annual and perennial types, so they too must be investigated. Oviposition behavior of adult females may also provide additional information as to the abundance of *L. maculicollis* larvae.

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CHOICE TESTS

With weevils





NO-CHOICE TESTS



Figure 2.1. Choice and no-choice plot description at the University of Massachusetts Turfgrass Facility in South Deerfield, MA. Five grass types were tested in these studies: *Poa annua*, creeping bentgrass types 3way and L93, Kentucky bluegrass, and Kentucky bluegrass/perennial ryegrass mixture.



Figure 2.2. Mean number of *Listronotus maculicollis* larvae \pm SE collected from five grass types (PA = *Poa annua*, 3W = creeping bentgrass type. 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) in low stress plots behind a green in 2000.



Figure 2.3. Percentage of nitrogen, phosphorus, and potassium in each of the five grass types (PA = *Poa annua*, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) in low stress plots in 2000. Data represent mean percentage of nutrients in combined grass plots \pm SE. Based on ANOVA, we found differences in the percentages of nitrogen (F _{4, 23} = 13.9, P < 0.001), and (*) indicates percent nitrogen in PA plots was significantly lower than remaining grasses when we compared means.



Figure 2.4. Mean number of *Listronotus maculicollis* larvae \pm SE in five different grass types (PA = *Poa annua*, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) with two different fertilization regimes in 2001 and 2002.







Figure 2.5. Mean weight of *Listronotus maculicollis* larvae collected from low stress, fertilizer plots in 2001 and 2002. Weights (mg) are means for each grass type \pm SE. An asterisk indicates larvae found in *Poa annua* (PA) plots weighed significantly more than larvae in creeping bentgrass type 3way (3W) and creeping bentgrass cv. L93 (L93) (Tukey's Pairwise Comparison, P < 0.05).



Grass Type

b

С



Figure 2.6. Percentage a) nitrogen, b) phosphorus, and c) potassium in each of the five grass types ($PA = Poa \ annua$, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) in low stress, fertilizer plots, 2001. Data represent mean percentage of nutrients in grass plots \pm SE.



Figure 2.7. Mean number of *Listronotus maculicollis* larvae in five grass types (PA = *Poa annua*, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) with two mowing heights \pm SE. Grasses mowed to short heights = 1.27 cm and grasses mowed to long heights = 3.81 cm.



Figure 2.8. Mean larval weights of *Listronotus maculicollis* collected from five grass types (PA = *Poa annua*, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93) ± SE in 2001 and 2002. Grasses mowed to short heights = 1.27cm and grasses mowed to long heights = 3.81cm.









Figure 2.9. Percentage a) nitrogen, b) phosphorus, and c) potassium in each of the five grass types (PA = *Poa annua*, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93) in mowing height plots, 2001. Data represent mean percentage of nutrients in grass plots ± SE.

a

b

С



Figure 2.10. Mean number of *Listronotus maculicollis* larvae collected from five different grass types (PA = *Poa annua*, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) \pm SE in A) choice test and B) no-choice test. ANOVA results imply significant differences between the grass types of A (F _{4, 24} = 17.54; P < 0.001) and B (F _{4, 24} = 16.77; P < 0.001), and when means were compared, an asterisk (*) indicates significantly more larvae were collected from PA plots in both A and B.

A



Figure 2.11. Mean number (\pm SE) of *Listronotus maculicollis* larvae collected from five creeping bentgrass cultivars (3W = type 3way, L93 = cv. L93, PC = cv. Penncross, PT = cv. Penntrio, and SS = cv. Southshore) in 2001 and 2002.

Table 2.1. Analysis of variance (ANOVA) with block, year, grass type, and fertilizer treatments as factors affecting the mean number of *Listronotus maculicollis* larvae in low-stress golf course turf plots in Yonkers, NY.

Factors	MS	df	Р	
Block	1.04	0.14	0.967	
Year	24.01	3.4	0.069	
Grass	80.37	11.37	< 0.0001	
Fertilization (+/-)	37.21	5.26	0.024	
Year*Grass	24.34	3.44	0.012	
Year* Fertilization	1.69	0.24	0.626	
Grass*Fertilization	5.54	0.78	0.54	
Year*Grass*Fertilization	0.87	0.12	0.974	

Table 2.2. Turf quality ratings from low-stress fertilizer study, 2002. Quality ratings were assessed for plots of five grass types ($PA = Poa \ annua$, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture).

Treatment	Sample	T	urf rating ± SE
		+ fertilizer	- fertilizer
Week 1	PA	5.5 ± 0.27^{ab}	5.5 ± 0.47 ^{ab}
	3W	5.0 ± 0.47 ^a	4.7 ± 0.45 ^a
	L93	5.7 ± 0.37 ^{ab}	5.5 ± 0.52 ^{ab}
	KB	6.6 ± 0.24 ^{ab}	6.3 ± 0.25 ^{ab}
	KB/PR	6.9 ± 0.19 ^b	7.0 ± 0.16 ^b
Week 2	PA	5.4 ± 0.27 ^a	5.5 ± 0.47 ^a
	3W	4.8 ± 0.34 ^a	4.8 ± 0.67 ^a
	L93	5.4 ± 0.30^{a}	5.1 ± 0.46^{a}
	KB	6.2 ± 0.30^{ab}	6.0 ± 0.22 ^{ab}
	KB/PR	7.5 ± 0.01 ^b	7.2 ± 0.22 ^b
Week 3	PA	5.7 ± 0.12^{a}	5.6 ± 0.29^{a}
	3W	5.2 ± 0.20^{a}	5.1 ± 0.89^{a}
	L93	6.2 ± 0.34^{ab}	6.2 ± 0.25 ^{ab}
	KB	6.3 ± 0.30^{ab}	6.3 ± 0.30^{ab}
	KB/PR	7.3 ± 0.12 ^b	7.3 ± 0.14^{b}
Week 4	PA	5.8 ± 0.34^{a}	5.5 ± 0.22^{a}
	3W	5.1 ± 0.33^{a}	6.3 ± 0.34 ^{ab}
	L93	5.5 ± 0.27^{a}	6.5 ± 0.16^{ab}
	KB	6.1 ± 0.19^{ab}	6.0 ± 0.22^{ab}
	KB/PR	7.2 ± 0.12 ^b	7.1 ± 0.19 ^b
Week 5	PA	5.5 ± 0.22 ^a	5.6 ± 0.29^{a}
	3W	5.4 ± 0.19^{a}	5.6 ± 0.40^{a}
	L93	5.6 ± 0.10^{a}	6.0 ± 0.27^{a}
	KB	6.2 ± 0.25^{a}	6.0 ± 0.22^{a}
	KB/PR	7.1 ± 0.12^{ab}	7.2 ± 0.12^{b}

Turf qualities are mean quality rating \pm SE. Numbers followed by the same letter are not significantly different among grass types within each week. Turfgrass quality ratings were determined weekly from 1 May to 3 June 2002.

Table 2.3. Regression analyses of turfgrass quality ratings from low stress, fertilizer study, 2002. Quality ratings were assessed for plots of five grass types (PA = Poa annua, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) over the five weeks of the study (1 May to 3 June).

Grass Type	Treatment	Regression Analysis			
		MS	F	\mathbf{r}^2	Р
PA	+ FERTILIZER	0.08	0.18	0.8	0.68
	- FERTILIZER	0.25	0.38	1.6	0.55
			<u>-</u>		
3W	+ FERTILIZER	0.61	1.28	5.5	0.27
	- FERTILIZER	5.45	8.6	27.2	0.007
		<u> </u>			
L93	+ FERTILIZER	0.005	0.01	0.0	0.92
	- FERTILIZER	2.88	4.04	15.0	0.06
КВ	+ FERTILIZER	0.41	1.31	5.4	0.27
	- FERTILIZER	0.18	0.64	2.7	0.43
KB/PR	+ FERTILIZER	0.005	0.05	0.2	0.83
	- FERTILIZER	0.045	0.45	1.9	0.51

Table 2.4. Analysis of variance (ANOVA) with block, year, grass type, and mowing height treatments as factors affecting the mean number of *Listronotus maculicollis* larvae in golf course turf plots in Yonkers, NY.

Factors	MS	df	Р
Block	5.54	3.03	0.023
Year	1.00	0.50	0.483
Grass species	5.54	3.30	0.0002
Mowing Ht.	4.14	2.46	0.045
Year*Grass species	5.18	2.57	0.044
Year* Mowing Ht.	0.04	0.02	0.888
Grass species*Mowing Ht.	7.87	2.79	0.075
Year*Grass species*Mowing Ht.	0.17	0.08	0.988

Table 2.5. Turf quality ratings from mowing height study, 2002. Quality ratings were assessed for plots of five grass types ($PA = Poa \ annua$, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture).

Treatment	Sample	ple Turf ratio		
		short	long	
Week 1	РА	5.6 ± 0.49^{a}	6.5 ± 0.34^{a}	
	3W	5.3 ± 0.30^{a}	6.2 ± 0.20^{a}	
	L93	5.4 ± 0.33^{a}	6.2 ± 0.29^{a}	
	KB	5.4 ± 0.43^{a}	6.9 ± 0.29^{a}	
	KB/PR	5.6 ± 0.43 ^a	7.4 ± 0.22^{a}	
Week 2	РА	5.3 ± 0.30^{a}	6.2 ± 0.34^{a}	
	3W	5.2 ± 0.41^{a}	6.1 ± 0.49^{a}	
	L93	5.5 ± 0.22^{a}	5.6 ± 0.36^{a}	
	KB	6.0 ± 0.39^{a}	6.6 ± 0.66^{a}	
	KB/PR	6.1 ± 0.22^{a}	6.9 ± 0.38^{a}	
Week 3	PA	5.4 ± 0.30^{a}	6.3 ± 0.20^{ab}	
	3W	$6.0 \pm 0.22^{\text{ ab}}$	5.4 ± 0.24^{a}	
	L93	$6.0 \pm 0.27^{ m ab}$	6.0 ± 0.22 ^{ab}	
	KB	6.2 ± 0.34^{ab}	6.7 ± 0.30^{ab}	
	KB/PR	6.6 ± 0.19 ^b	7.3 ± 0.12^{b}	
Week 4	PA	5.4 ± 0.43^{a}	5.8 ± 0.25^{a}	
	3W	5.2 ± 0.12^{a}	5.4 ± 0.29^{a}	
	L93	5.2 ± 0.12^{a}	5.5 ± 0.35^{a}	
	KB	5.6 ± 0.51^{a}	6.6 ± 0.25^{ab}	
	KB/PR	6.0 ± 0.27 ^a	7.3 ± 0.12 ^b	
Week 5	PA	5.6 ± 0.43^{a}	6.1 ± 0.19^{a}	
	3W	5.8 ± 0.25^{ab}	5.7 ± 0.48 ^a	
	L93	6.1 ± 0.24^{ab}	5.7 ± 0.20^{a}	
	KB	6.3 ± 0.67^{ab}	6.8 ± 0.12^{ab}	
	KB/PR	6.7 ± 0.48 ^b	7.2 ± 0.12^{b}	

Turf qualities are mean quality rating \pm SE. Numbers followed by the same letter are not significantly different among grass types within each week based on comparison of means. Turfgrass quality ratings were determined weekly from 1 May to 3 June 2002

Table 2.6. Regression analyses of turfgrass quality ratings from mowing height study, 2002. Quality ratings were assessed for plots of five grass types (PA = Poa annua, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) over the five weeks of the study (1 May to 3 June).

Grass Type	Treatment	Regres	5		
		MS	df	\mathbf{r}^2	Р
PA	SHORT	0.005	0.01	0.0	0.93
	LONG	0.72	2.9	11.2	0.10
3W	SHORT	0.72	1.38	5.7	0.25
	LONG	1.46	3.29	12.5	0.08
L93	SHORT	0.84	1.65	1.65	0.21
	LONG	0.61	1.48	6.1	0.24
KB	SHORT	0.20	0.03	0.1	0.87
	LONG	0.02	0.03	0.1	0.86
KB/PR	SHORT	0.61	1.34	5.5	0.26
	LONG	0.00	0.00	0.0	1.0

Table 2.7. Turf quality ratings from choice test, 2002. Quality ratings were assessed for plots of five grass types ($PA = Poa \ annua$, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture).

Sample	Treatment	7	Turf rating ± SE
		+ weevils	- weevils
Week 1	PA	6.3 ± 0.2	7.0 ± 0.16
	3W	7.2 ± 0.12	7.6 ± 0.19
	L93	6.5 ± 0.16	6.9 ± 0.1
	KB	6.7 ± 0.34	6.8 ± 0.2
	KB/PR	7.6 ± 0.24	7.5 ± 0.32
Week 2	PA	6.6 ± 0.33	7.7 ± 0.34
	3W	7.2 ± 0.12	7.6 ± 0.19
	L93	6.8 ± 0.16	7.1 ± 0.19
	KB	6.8 ± 0.2	6.7 ± 0.25
	KB/PR	7.1 ± 0.19	7.1 ± 0.24
Week 3	PA	7.7 ± 0.2	8.1 ± 0.1
	3W	7.0 ± 0.22	7.0 ± 0.16
	L93	6.8 ± 0.2	6.8 ± 0.25
	KB	7.0 ± 0.16	6.7 ± 0.25
	KB/PR	6.9 ± 0.22	6.9 ± 0.24
Week 4	РА	6.3 ± 0.12	6.9 ± 0.19
	3W	6.6 ± 0.1	6.7 ± 0.12
	L93	6.6 ± 0.1	6.6 ± 0.1
	KB	6.5 ± 0.16	6.3 ± 0.12
	KB/PR	6.7 ± 0.2	6.4 ± 0.1
Week 5	PA	5.1 ± 0.37	6.7 ± 0.25
	3W	6.3 ± 0.2	6.6 ± 0.24
	L93	6.3 ± 0.3	6.0 ± 0.0
	KB	6.8 ± 0.2	6.4 ± 0.24
	KB/PR	6.8 ± 0.12	6.8 ± 0.25
Week 6	PA	$4.1 \pm 0.33^*$	6.7 ± 0.25
	3W	6.7 ± 0.2	6.9 ± 0.1
	L93	6.8 ± 0.25	6.1 ± 0.22
	KB	6.8 ± 0.25	6.9 ± 0.1
	KB/PR	6.7 ± 0.12	7.2 ± 0.2

An asterisk (*) indicates significantly lower turf quality (Tukey's Pairwise Comparison, P < 0.001) in PA with weevils compared to PA without weevils. Grass quality ratings were taken weekly on all turf cores, from 6 May until the termination of the experiment on 15 June.

Table 2.8. Regression analyses of turfgrass quality ratings from choice tests at the Turfgrass Research Facility in South Deerfield, MA, 2002. Quality ratings were assessed for plots of five grass types ($PA = Poa \ annua$, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) over the six weeks of the study (6 May to 15 June).

Grass Type	Treatment	Regression Analysis			
		MS	df	\mathbf{r}^2	Р
PA	+ WEEVILS	22.6	22.9	44.9	< 0.001
	- WEEVILS	2.3	5.23	15.7	< 0.001
3 W	+ WEEVILS	2.0	13.3	32.1	0.001
	- WEEVILS	2.24	13.2	32.0	0.001
L93	+ WEEVILS	0.003	0.01	0.0	0.91
	- WEEVILS	4.02	31.9	53.3	< 0.001
KB	+ WEEVILS	0.00	0.00	0.0	1.0
	- WEEVILS	0.046	0.20	0.7	0.66
KB/PR	+ WEEVILS	2.24	10.95	28.1	0.003
	- WEEVILS	0.60	1.74	5.9	0.20

Table 2.9. Turf quality ratings from no-choice test, 2002. Quality ratings were assessed for plots of five grass types ($PA = Poa \ annua$, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture).

Sample	Treatment	$\underline{\qquad} Turf rating \pm SE$
Week 1	PA	6.3 ± 0.25^{a}
	3W	7.1 ± 0.19 ^a
	L93	6.9 ± 0.33 ^a
	KB	6.5 ± 0.5 ^a
	KB/PR	7.2 ± 0.49^{a}
Week 2	PA	6.6 ± 0.33^{a}
	3W	7.2 ± 0.12 ^a
	L93	6.8 ± 0.16 ^a
	KB	6.5 ± 0.16 ^a
	KB/PR	6.4 ± 0.24 ^a
Week 3	PA	6.4 ± 0.24 ^a
	3W	6.4 ± 0.19 ^a
	L93	6.2 ± 0.12 ^a
	KB	6.3 ± 0.37 ^a
	KB/PR	6.7 ± 0.2 ^a
Week 4	PA	5.2 ± 0.25 ^a
	3W	6.1 ± 0.24 ^b
	L93	5.8 ± 0.12 ^b
	KB	6.2 ± 0.2 ^b
	KB/PR	6.6 ± 0.1 ^b
Week 5	PA	3.5 ± 0.39^{a}
	3W	6.9 ± 0.24 ^b
	L93	6.4 ± 0.19 ^b
	KB	6.8 ± 0.2 ^b
	KB/PR	6.9 ± 0.19 ^b
Week 6	PA	2.0 ± 0.84^{a}
	3W	6.8 ± 0.25 ^b
	L93	6.2 ± 0.2 ^b
	KB	7.0 ± 0.0 ^b
	KB/PR	7.0 ± 0.16^{b}

Turf qualities are mean quality rating \pm SE. Numbers followed by the same letter are not significantly different from each other within each week. Grass quality ratings were taken weekly on all turf cores, from 6 May until the termination of the experiment on 15 June.

Table 2.10. Regression analyses of turfgrass quality ratings from no-choice tests at the Turfgrass Research Facility in South Deerfield, MA, 2002. Quality ratings were assessed for plots of five grass types ($PA = Poa \ annua$, 3W = creeping bentgrass type 3way, L93 = creeping bentgrass cv. L93, KB = Kentucky bluegrass, and KB/PR = Kentucky bluegrass/perennial ryegrass mixture) over the five weeks of the study (6 May to 15 June).

Grass Type	Treatment	Regression Analysis			
		MS	df	r^2	P
PA	+ WEEVILS	69.1	52.2	65.1	<0.001
3W	+ WEEVILS	0.026	0.08	0.3	0.78
L93	+ WEEVILS	1.45	5.68	16.9	0.024
КВ	+ WEEVILS	0.78	2.94	9.5	0.97
KB/PR	+ WEEVILS	0.11	0.03	0.1	0.86

CHAPTER 3

LISTRONOTUS MACULICOLLIS: SPRING EMERGENCE FROM OVERWINTERING SITES AND DISTRIBUTION ON TURFGRASS HOSTS

<u>Abstract</u>

Golf course superintendents often report poor quality turfgrass on the perimeters of their short-mowed turf (primarily fairways) from *Listronotus maculicollis* (Dietz) larval feeding. In this study, we detected a trend of lower quality in fairway edges, but distribution of *L. maculicollis* larvae was even across the width of a fairway. These preliminary results suggest that an unknown factor may contribute to damage observed on short-mowed grass edges. Based on the distribution of adults, we concluded that they move considerable distances across golf course turf. We also investigated the spring emergence behavior of adult weevils, and we concluded that weevils emerge from overwintering sites in the spring and walk to suitable turfgrass hosts. Based on these results, perimeter control applications to immigrating adults warrants future evaluation.

Introduction

L. maculicollis is a recurring golf course pest that many superintendents in the Northeast must manage annually. Most management strategies consist of applying an insecticide to large areas of short-mowed turfgrass such as greens, tees, and fairways. Spring chemical control targeting emerging adults is currently the most reliable
alternative to manage damaging numbers of *L. maculicollis* larvae (Vittum 1999). Golf course superintendents with persistent weevil populations apply between two and five chemical treatments per season (Cameron and Johnson 1971a, Vittum 1999).

Adults overwinter in areas adjacent to golf course turf (Cameron and Johnson 1971b, Vittum 1980, Vittum and Tashiro 1987, Vittum et al. 1999). Vittum (1980) found that white pine litter provided optimal overwintering habitat for weevils. In spring, adult weevils move out from hibernation sites onto the highly maintained turfgrass to oviposit. Vittum (1980) reported that*L. maculicollis* adults travel through rough-mowed turf to fairway edges, where ovipositon principally takes place.

Based on this information, we would expect the spring distribution of L. *maculicollis* adults and larvae to be concentrated primarily on perimeters of fairways, tees, and greens nearest to adult overwintering sites. In fact, Vittum (1980) documented that L. maculicollis spring infestations are concentrated on fairways bordering overwintering sites. Furthermore, golf course superintendents observe most L. maculicollis damage in the spring on perimeters of their highly maintained areas, especially fairways nearest weevil overwintering (H. Watson, personal communication). Many superintendents are convinced female weevils walk from overwintering sites until they reach the first suitable oviposition site (H. Watson, personal communication). Plum curculio adults, Conotrachelus nenuphar Herbst, exhibit this type of 'first stop' emergence behavior; most adults climb the first available apple tree to oviposit, and these accessible trees are most commonly located in perimeter rows of the orchards (Racette et al. 1990, Chouinard et al. 1993, Chouinard et al. 1994). If L. maculicollis adults emerge from hibernation and exhibit a similar type of first-stop behavior, we would anticipate

primary oviposition on fairway, tee, or green edges. Based on observations of adult weevil emergence and oviposition, most northeastern golf course managers attribute their perimeter damage, observed in June, to first generation *L. maculicollis* larval feeding, and fairway perimeters closest to adult weevil overwintering sites are likely candidates for damage.

Research has shown other turfgrass pests to preferentially damage edges of highly maintained turf. *Ataenius spretulus* Haldeman, *Aphodius granarius* L., *Papillia japonica* Newman, and *Rhizotrogus majalis* Razoumowsky attacked short-mowed grass more often than long-mowed grass, and in each case, most damage was reported on fairway perimeters (Smitley et al. 1998, Rothwell and Smitley 1999, and Smitley, unpublished data).

Other coleopteran species have been reported to infest edges of host plant stands. Damage caused by strawberry bud weevil, *Anthonomus signatus* Say, occurs primarily on boundaries of strawberry fields (Kovach et al. 1993). Mexican bean beetle, *Epilachna varvestis* Mulsant, egg masses were concentrated on field edges closest to adult overwintering sites (Barrigossi et al. 2001). One third of *A. pomorum* L., apple blossom weevil, populations remained on the first encountered apple tree after spring emergence; a strong edge effect was noted with high weevil populations at orchard perimeters compared with orchard centers (St. Toepfer and Dorn 1999). LeBlanc et al. (1984) reported elevated plum curculio damage on orchard borders. In controlled studies, *Phyllotreta cruciferae* Goeze, flea beetles, have also been reported to feed more readily on crucifers located at arena edges (Palaniswamy and Lamb 1992).

In addition to observations indicating L. maculicollis oviposition preference for fairway perimeters, adults are believed to walk to these egg-laying sites. However, Cameron and Johnson (1971b) and Vittum et al. (1999) have reported L. maculicollis flight. In fact, Vittum (1980) captured flying adults in black light traps on golf course roughs during spring and summer months. Cameron and Johnson (1971b) and Schread (1970) also caught flying weevils on plywood rectangles coated with yellow paint and sticky material. Cameron (1970) found more developed wing muscles in adult weevils in spring and in late June than other parts of the year, but only a small percentage, 30%, of the population had well-developed wing muscles during these peak times. Vittum (1980) also reported a small number of weevils developed flight muscles in her field studies. Although Vittum (1980) noted flight muscle development, she defended the supposition that L. maculicollis walk onto golf course turf to oviposit in spring, based on visual observations of walking adults. Nevertheless, details of spring emergence and primary location of emerging weevil oviposition need to be clarified.

Knowledge of *L. maculicollis* oviposition behavior is fundamental to implementing control strategies appropriate for first generation larvae. If oviposition is indeed restricted to the perimeters of fairways perimeter chemical applications could replace the whole fairway spray approach currently employed on northeastern golf courses (Rothwell, personal observation) and reduce the area treated, at least in the spring. Chouinard et al. (1992) found that orchard border sprays for plum curculio control reduced damage to apples from 57% to 2.4%. Perimeter sprays have been suggested for *L. maculicollis* control, but the efficacy has not been tested. We must first determine the mode of *L. maculicollis* adult spring emergence. If emerging adults do

walk onto host plants, chemical barrier strips established in the roughs between overwintering and oviposition sites might be an alternative control measure.

In this study, we will investigate aspects of *L. maculiocollis* seasonal activity and distribution on golf course turf: 1) *L. maculicollis* adult and larval distribution on golf course turf through the first generation, 2) the quality of the host plant (golf course turf) in relation to distribution of larvae, 3) adult spring emergence behavior, and 4) *L. maculicollis* population dynamics from year to year. We hope to use this information to recommend golf course management strategies to prevent *L. maculicollis* damage.

Methods and Materials

Weevil distribution and presence. Adult *L. maculicollis* were monitored weekly at Hartford Golf Club, Hartford, CT in 2000, 2001, and 2002. The fairway where we conducted this research was based on previous knowledge of past *L. maculicollis* infestation. We also were aware of the known weevil overwintering site on the east side of the fairway that bordered the test location. In addition, white pine trees lined the west side of the fairway, and these trees have been determined to be the favored *L. maculicollis* overwintering site (Vittum 1980). Therefore, the potential for *L. maculicollis* infestation from both sides of our chosen fairway was high.

Five transects were laid out, with three m in between each, across the fairway width, which was approximately 25.6 m wide (Figure 3.1). Each transect was sectioned off into 14 1.8 m x 0.9 m plots. Individual plots of each transect were sampled for adult *L. maculicollis* using a modified leaf blower (Homelite[®], Anderson, SC, d25mhv, UT08084) that vacuumed insects from the turf surface (Figure 3.2). A wire mesh screen

(natural aluminum 0.011 gauge wire, 18 x 16 mesh count) prevented weevils from entering the motor of the blower when they were sucked off the turf through the funnel. This screen was easily detachable from the funnel, which enabled us to count weevils and remove grass and other debris sucked up by the vacuum. The vacuum was only capable of retrieving adult weevils from the surface of short-mowed turf--fairways, tees, and greens. Weevils cannot be suctioned from grass mowed to rough height (>3.81 cm) (Rothwell, unpublished data).

During the 2000 season, each plot was vacuumed in a randomized fashion for 10 s from 5 May to 7 June. In 2001, we extended the sampling period and vacuumed adult weevils from 27 April to 2 November. The following year, we sampled for adult weevils only through the first generation: 26 April through 10 June. Adult *L. maculicollis* were counted and returned to the specific plots from which they had been removed. For all three seasons, adult samples were made weekly, between 0700 and 1000 on sunny days.

L. maculicollis larvae were collected from the transects with a 5.4 cm²-diameter turf-corer (Turf-Tec International[®], Oakland Park, FL) once per season. Each year, five cores were removed from each plot in all five transects. Turf plugs were returned to the laboratory in Amherst, MA where they were hand-inspected and all larvae were counted and recorded.

Turf Quality Ratings. Since 2000 and 2001 yielded no differences in location of *L*. *maculicollis* across the width of the fairway, but we observed grass on fairway perimeters to be of lower quality than grass in the fairway center in 2001, we evaluated the quality of the turfgrass in each plot weekly in 2002. The National Turf Evaluation Program (NTEP) turf measurement scale was used (Turgeon 1980). We assessed the turf quality visually, where a quality score of 1 = unacceptable turf quality (dead) and a score of 9 = uniform, green grass. Each quality rating was based on a combination of color, density, and uniformity of the grass. Turf ratings were taken between 0700 and 1000 on sunny days beginning 6 May and ending 6 June.

Spring Emergence Behavior. To determine the method of *L. maculicollis* adult emergence in the spring, we erected large Plexiglas[®] cubes fitted with pitfall traps in rough-mowed turf, adjacent to a fairway at Hartford Golf Club, Hartford, CT on 8 March 2002. The cubes were constructed from four 0.914 m x 0.914 m sheets of 3.2 mm clear Plexiglas[®] (Figure 3.3). The Plexiglas[®] panes were connected with 3.5 mm angle irons to form a square, and the four inner surfaces of the Plexiglas[®] were coated with sticky insect trap material (Tangle-Trap[®], Grand Rapids, MI). Cubes were secured with roping to prevent collapse from strong winds, and the tops were covered with a thin white netting to prevent weevils from escaping the enclosure by upward flight.

Two pitfall traps were positioned inside the cubes on the north and south facing sides. These traps were parallel to the length of the golf course fairway, which provided maximum capture potential for weevils traveling from overwintering sites toward fairway turf. Pitfall traps were constructed of 1.2 m polyvinyl chloride (PVC) piping with a 5.4 cm-diameter (Figure 3.4). Ten 3.2 cm holes were drilled along the length of the piping with a 7.0 mm space between the centers of each hole. Funnel-shaped plastic cups, with bottoms removed, were inserted in the holes and secured with waterproof caulking. Two traps were placed in the ground, inside the cube on opposite sides, with the tops of the

cups flush with the turf surface. One end of each trap was sealed and the other (unsealed) end of the trap was attached to a 3.8 L plastic jug. Adult weevils captured in the pitfall traps walked or were flushed into the jug, where they were collected. Traps were installed in the ground with a slight slope so water drained into the jug.

We placed 60 adult *L. maculicollis*, collected from the nearby overwintering site, inside each cube. Weevils were positioned directly in the center of the grassy portion of the cube, equidistant from the cube sides and pitfall traps. The cubes were monitored weekly from 15 March to 15 May 2002; sticky cube sides were inspected for insects that had been ensnared in the sticky material and collection jugs were monitored for pitfall trap-captured weevils. We presumed weevils that would have flown onto the golf course would be captured on the sticky-coated sides of the cube while walking weevils would be caught in the ground-level pitfall traps.

Pitfall Traps. In 2000, 2001, and 2002, we monitored the timing of adult weevil emergence from overwintering sites at Hartford Golf Club. Two pitfall traps, as described above, but longer in length, were placed parallel to golf course fairways in a rough-mowed (>3.81 cm) area of the golf course, near a known *L. maculicollis* overwintering site. Traps were constructed of 3.05 m PVC piping with a 5.4 cm-diameter. Again, holes were drilled into the pipe and filled with bottomless plastic funneled cups. One end of the trap was sealed, and the other end was fitted with a plastic container to hold captured emerging weevils. Two traps were placed in the overwintering area, with the cups level with the turf surface. Traps were again placed on a slight incline to encourage weevils to walk into the collection jug and for water to drain properly when

flushing the traps. Pitfall traps were examined twice weekly from 1 March through 5 May for all years.

Statistical Analysis. Plots 1 and 2 were the closest plots to L. maculicollis preferred overwintering sites whereas plots 13 and 14 were farthest from these sites. Because so few weevils were recovered from individual plots throughout the study, data from adjacent plots were pooled: plots 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, and 13 and 14. Mean number of L. maculicollis adults was analyzed with year, week, and plot number as factors in a parametric 3-way analysis of variance (ANOVA). Because we sampled the plots in the transects repeatedly throughout the season, we used a repeated measures ANOVA. A correlation analysis was employed to measure the relationship between locations of L. maculicollis adults and larvae. Turf quality measurements were analyzed with ANOVA with week and plots as factors. Differences among these all treatment means were tested using Tukey's Pairwise Comparison at $\alpha = 0.05$. To compare mean number of weevils captured in the pitfall traps and on the sides of the sticky cube, we used a one-way analysis of variance. The software Minitab[®] (2000) was used for correlation analysis and the one-way ANOVA. For the repeated measures analysis, we used the statistical package SAS[®] (1997).

<u>Results</u>

Weevil distribution and presence. Throughout the three-year study, the weevil population at Hartford Golf Club varied significantly among years (F $_{2, 125} = 25.68$; P < 0.001, Figure 3.5), and we collected more adults in 2000 (Tukey's Pairwise Comparison

(< 0.05). The number of larvae collected was also significantly different between years of the study (F $_{2, 41} = 3.75$; P = 0.032, Figure 3.5). Comparison of means showed that 2000 had more larvae (P < 0.05).

When we investigated the distribution of adult L. maculicollis, we found no significant differences between the plots across the width of the fairway in any of the three years (F $_{18, 125} = 1.17$; P = 0.303, Figure 3.6a, b, c). Adults appear to move across all plots over the fairway width, and no distinct pattern could be discerned. We collected larvae from all plots across the fairway width in all years of the study (Figure 3.7). In 2000, we detected larvae throughout the fairway, but we did not make any qualitative assessment of turf perimeters. In 2001 larvae were evenly distributed across the fairway width (F $_{13, 69} = 1.48$; P = 0.134), but noticeable damage was observed on fairway perimeters. The second-generation larvae in 2001 appeared to favor the center of the fairway rather than the fairway edges, but too few larvae were collected to perform statistical analysis (Figure 3.8). In 2002, we saw no significant differences in distribution of larvae across fairway plots (F $_{13, 69} = 0.62$; P = 0.831). Using Pearson's correlation analysis, we did not detect a relationship between adult and larval locations in any year of the study (r = -0.116, P = 0.0465; r = 0.221, P = 0.160; r 0.263, P = 0.92 for 2000, 2001, and 2002, respectively).

Although we could not distinguish between the distribution of adult weevils across fairway turf, we did observe significant differences between the numbers of adults at different weeks of the study (F $_{15, 125} = 4.31$; P < 0.001). Fewer weevils were collected on the first sample dates in all years; these dates were 6 May 2000, 27 April 2001, and 26 April 2002. In 2000, we noted an increase in adult weevils on 18 and 24 May (F $_{5, 41} =$ 4.74; P = 0.002) compared with the first week of the study. We also detected a 'peak activity' of adult weevils, which occurred on 30 May 2002 (F _{5,41} = 4.59; P = 0.002).

Turf Quality Ratings. Turf quality decreased slightly over the duration of the experiment. More importantly, we observed that turfgrass quality varied between plots (F $_{6, 34} = 2.29$; P = 0.063). When we compared mean turfgrass qualities for plots 1 and 2, which are nearest *L. maculicollis* overwintering sites, they showed a trend of poorer quality than remaining transect plots (Figure 3.9).

Pitfall Traps. We captured weevils in pitfall traps throughout the spring season for all years of the study (Table 3.2). Early emerging adult *L. maculicollis* were caught in the pitfall traps on 24 March in 2000, 5 April in 2001, and 7 April in 2002. Pitfall trap catches continued throughout April in all years and in 2001, we captured weevils on 5 May. In all years, we saw an increase in adult activity after the first sampling date, and in 2002 we had a peak activity of adults on 30 May, but overall, we did not observe a mass spring emergence in any of the three years of the study; weevils appear to emerge from overwintering sites throughout the duration of the spring season although some dates had higher collection rates than other dates. This staggered spring emergence is inconsistent with the apparent mass emergence of second-generation adults reported in June 2001 (Figure 3.10).

Spring Emergence Behavior. We captured 26 adults in the pitfall traps compared with only two *L. maculicollis* adults ensnared on the sticky sides of the cube (Figure 3.11). Since significantly more weevils were captured in the pitfall traps ($F_{1,11} = 16.41$; P = 0.002) than the sticky cube sides, our data indicate adult *L. maculicollis* emerge from overwintering sites in the spring and tend to walk onto host plants rather than fly.

Discussion

Although observational evidence suggests that first generation *L. maculicollis* damage is restricted to fairway perimeters due to higher numbers of larvae on these edges, our results from our test fairway do not support these observations. We did, however, detect a trend of poorer turf quality on the perimeters, but this damage does not appear to be caused by higher numbers of larvae feeding in this region. From three years of collected data, both adult and larval distribution appears to be fairly uniformly distributed across the width of a highly manicured fairway.

Based on our results, we confirm that *L. maculicollis* adults are highly mobile on highly maintained turf, as evident by lack of a distribution pattern across the fairway width. More intense sampling, perhaps daily, may have provided a more comprehensive delineation of the distribution of spring emerging adults on fairway turf. Prestige et al. (1985) found sampling of *L. bonariensis* (Kuschel) adults to be difficult and inaccurate because of high adult mobility. We, too, may have imprecision of adult distribution based on *L. maculicollis* ability to move great distances. In addition, because *L. maculicollis* adults are capable of covering such distances, we can only show a snapshot of the distribution on any given day. To confirm our preliminary *L. maculicollis* adult

distribution pattern, a mark-recapture study must be performed to monitor individual weevils and their movement across fairway widths. A preliminary mark-recapture study was conducted in 2001 (Rothwell, unpublished data) in outdoor cages where we marked over 5000 weevils and released them in enclosed arenas, but we recaptured less than 2% of weevils. Regardless of overall adult distribution, if females have a tendency to oviposit as soon as they reach short-mowed grass (as suggested by Vittum 1980), then overall adult distribution is not as crucial from a management standpoint as are oviposition habits that result in subsequent larval distribution.

St. Toepfer and Dorn (1999) reported that apple blossom weevil, A. pomorum, dispersal and orchard colonization were dependent on temperature. In our studies, temperature may have played a similar role in adult *L. maculicollis* distribution across the fairway. We monitored adult movement in the laboratory at a range of temperatures and determined that L. maculicollis possess the capability to travel considerable distances in a short amount of time under varying temperatures. We found overwintering adult weevils held at 20° C for 24 hours covered 81.3 ± 17.7 cm in five minutes (Rothwell, unpublished data), and even adults held at 0° C were capable of moving 26.9 ± 6.4 cm in the same amount of time. Our field sampling method may not have monitored exact distribution because weevil mobility is influenced by temperature. For example, if samples were collected on a warm morning, early emerging adults may have walked through the perimeter plots into the center plots of the fairway before samples were taken. In addition to establishing that adult weevils can walk considerable distances, we also confirmed that adult weevils emerging from overwintering sites in the spring are more likely to walk to oviposition sites than fly. Since emerging weevils walk through the

rough-mowed turf to reach the fairway, perimeter sprays in the spring may potentially provide control for adult *L. maculicollis*. Perimeter control for plum curculio infested orchards only showed a moderate reduction with one spray, but when two applications were applied to orchard edges, results provided economically acceptable control (Chouinard et al. 1992). Similar research needs to be done to determine whether perimeter sprays are a viable option for *L. maculicollis* control. However, considering the adults' significant capacity to travel great distances as well as their variation in emergence times, a multiple perimeter spray approach may be appropriate. Regardless, a perimeter spray will result in markedly less insecticide use compared to the currently employed 'wall to wall' spray approach. A fairway perimeter treatment applied with a 6.1 m spray boom would cover the approximate area of 1486.6 m², whereas spraying an entire fairway would require coverage of about 5665.6 m², assuming an average of 101, 171 m² of fairway on a given golf course.

Although most chemical applications target spring emerging adult weevils, *L. maculicollis* larvae in fairway turf are the damaging stage of this pest. From our study, larval distribution was uniform from the perimeter of the fairway to the center. However, these findings contradict the observations of fairway perimeter damage, which until now was thought to be caused solely by larval feeding. Even though *L. maculicollis* larvae were consistently distributed across the entire width of the fairway, we did indeed detect a trend of poor quality turf on the fairway edge nearest weevil overwintering. Thus, from our preliminary results, the compromised quality of fairway edges cannot fully be attributed to *L. maculicollis* feeding. Therefore, we speculate that reported damage, once credited exclusively to larval feeding, might be caused by a combination of larval feeding

and an unknown factor, presumably some agronomic or biotic stress. Additionally, according to golf course superintendents, *P. annua* encroaches on creeping bentgrass fairways at higher infestation levels along fairway edges (N. Rothwell, personal communication). Because this grass is more susceptible to environmental factors (Gibeault 1966, Beard 1982, Mitch 1998), these low quality edges may be attributed to this weak grass species in addition to larval feeding.

Other insects have reported uniform distributions on turfgrass hosts while damage to perimeters is consistently higher than other areas. Rothwell and Smitley (1999) and Smitley et al. (1998) observed A. spretulus and A. granarius larvae to be evenly distributed across golf course fairways in Michigan. However, reported damage from these insects was found to be greater on the fairway edges. Furthermore, Smitley (unpublished data) reported that *P. japonica* and *R. majalis* larvae were uniformly dispersed across fairway widths, but moisture levels, rather than larval feeding, most heavily influenced fairway turf quality. Damage caused by these scarab larvae was most visible on fairway perimeters where turf was under moisture stress, and feeding damage was compounded when turf lacked adequate moisture. Potentially, if superintendents increased water supplies to golf course perimeters, those areas could become more resistant to scarab larval feeding. Results of our L. maculicollis study have many similarities to the Rothwell and Smitley (1999) and Smitley (unpublished data) observations, which, in turn, may have implications for future turfgrass insect control.

However, our results are based on only one golf course fairway. Due to logistics, we were not able to include multiple fairways at our chosen golf course or fairways from

other golf courses. Therefore, to confirm our results, investigations of other fairway widths needs to be conducted to verify *L. maculicollis* larval distribution.

Lastly, our study shows a great fluctuation in *L. maculicollis* populations from year to year. These results reinforce standing recommendations for superintendents to assume an active role in monitoring pest activity on the golf course. Because *L. maculicollis* populations vary, we highly recommend aggressive scouting approaches for adult populations in early spring and again in late May to early June to monitor damaging larvae.

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Rough

Listronotus maculicollis preferred overwintering site

Figure 3.1. Transect plot diagram of fairway width at Hartford Golf Club, Hartford, CT.



Figure 3.2. Modified leaf blower (Homelite[®], Anderson, SC, d25mhv, UT08084) used to remove adult *Listrontous maculicollis* adults from the short-mowed turf surface.

Overwintering Site



0.9 m

Fairway

Figure 3.3. The Plexiglass[®] cube construction placed in a *Listronotus maculicollis* overwintering site on Hartford Golf Club, Hartford, CT. All sides of the trap are covered with a sticky material, and the pitfall traps were placed flush with the ground surface. These cubes were monitored weekly beginning 15 March 2002.



Figure 3.4. Linear pitfall trap placed at ground level to capture walking *Listronotus* maculicollis adults.



Figure 3.5. The number of *Listronotus maculicollis* \pm SE collected from transect plots at Hartford Golf Club for three years, 2000, 2001, and 2002. An asterisk (*) indicates weevils captured during this year was significantly different than the other two years (F ₂, ₁₂₅ = 25.68; P < 0.001 for adults captured and F _{2, 41} = 3.75; P = 0.032 for larvae captured).









Distance from nearest overwintering site

Figure 3.6. Adult *Listronotus maculicollis* distribution across a fairway width for years a) 2000, b) 2001, and c) 2002. Distances are measured from nearest weevil overwintering sites from the east side of the fairway.



Figure 3.7. Distribution of first generation *Listronotus maculicollis* larvae across the width of a golf course fairway 2000, 2001, and 2002. No significant differences exist between number of larvae between distances from fairway edge nearest overwintering site (F $_{13, 69} = 0.72$, P = 0.74in 2000; F $_{13, 69} = 1.48$, P = 0.134 in 2001; F $_{13, 69} = 1.46$, P = 0.164 in 2002).



Figure 3.8. Distribution of second-generation *Listronotus maculicollis* larvae across the width of a golf course fairway 2001. Too few larvae were recovered for statistical analysis.



Figure 3.9. Turfgrass quality ratings on plots in transects across fairway width 2002. Ratings are mean number \pm SE. Plots were rated weekly beginning 6 May. Plots 0-3.7 m, which are closest to *Listronotus maculicollis* overwintering sites showed a trend of a difference in quality than remaining plots (F _{6, 34} = 2.29; P = 0.0634).



Figure 3.10. Weekly adult *Listronotus maculicollis* collections from 27 April – 12 October 2001 at Hartford Golf Club. Weevils were collected with a modified leaf blower and each plot was vacuumed for 10 s. The peak correlates with approximate timing of second generation adult emergence.



Figure 3.11. Total spring emergence *Listronotus maculcollis* adult captures in 2002. Pitfall trap indicates weevils collected in 2 pitfall traps, and cube denotes weevils caught on the sticky sides of the Plexiglas[®] cube. Significant differences were detected between the number of weevils captured with the pitfall trap method and those trapped on the sticky cube sides (F_{1,11} = 16.41; p = 0.002). Weevils fly to reach the sticky sides of the cube, whereas weevils walk to enter the pitfall traps. Table 3.1. Pitfall catches of *Listronotus maculicollis* adults at Hartford Golf Club, Hartford, CT in spring of 2000, 2001, and 2002.

<u>2000</u>

Sample date	Number weevils
13 March	0
24 March	4
3 April	15
8 April	25
17 April	18
26 April	5
5 May	0

<u>2001</u>

Sample Date	Number weevils
25 March	0
5 April	3
12 April	9
19 April	16
26 April	5
5 May	2

Sample Date	Number weevils
25 March	0
1 April	0
7 April	2
14 April	1
21 April	4
28 April	0
5 May	0

CHAPTER 4

INVESTIGATION INTO *LISTRONOTUS MACULICOLLIS* ADULT MOVMEMENT, REPRODUCTIVE SEASONALITY, AND REPRODUCTIVE DIAPAUSE AND THEIR EFFECTS ON AN ACCURATE PREDICTION MODEL

Abstract

Adult movement was investigated to determine optimal timing for control measures against emerging spring populations of *Listronotus maculicollis* (Dietz) adults, which in turn reduce numbers of damaging larvae. Reproductive seasonality and reproductive diapause were investigated to better understand the seasonal history of *L. maculicollis*. We determined the optimal temperature for adult activity to be 20° C, and when used with phenological indicators, it will offer golf course superintendents more precise timing to apply control strategies in the spring season. In addition, we established the minimum temperature for *L. maculicollis* larval growth to be 13.3° C.

Superintendents can use this temperature in a degree-day equation to predict initial larval activity on golf course turf. From the three year study, we predict larvae will first be observed on the golf course between 150 DD and 200 DD. We charted reproductive seasonality of *L. maculicollis* and found that there are two generations per year. By monitoring reproductive organ growth of male and female weevils over a 12-month period, we propose that *L. maculicollis* undergo a reproductive diapause based on small size of reproductive organs during the winter months. Our preliminary results also show

that warmer temperatures and shorter daylength influences termination of reproductive diapause.

Introduction

L. maculicollis is a pest of golf course turf in the northeastern United States, where larvae damage the highly maintained and short-mowed grass of tees, greens, collars, and fairways. Golf course superintendents currently have no method of control other than chemical applications, and may treat infested areas two to five times per season, which can be costly (Cameron and Johnson 1971a, Vittum 1999).

L. maculicollis overwinter as adults in areas adjacent to the golf course (Cameron 1970, Vittum 1980, Vittum and Tashiro 1987). Throughout late March and April, adults emerge from hibernation sites and move onto short-mowed grass to oviposit within the leaf sheath of the grass plant (Cameron and Johnson 1971b). Because we do not have a precise time to measure adult movement, superintendents apply multiple sprays to target weevils as they emerge at variable times.

First and second instars feed on the inner blades of the grass plant (Cameron and Johnson 1971b, Vittum and Tashiro 1987), and migrate to the crown as they grow. Cameron (1970) reported that one *L. maculicollis* larva is able to kill as many as twelve grass plants during its larval development. Fifth (ultimate) instars cause the most severe damage, and are able to destroy large areas of turf. In the metropolitan New York area, damage to golf course turf is most often reported in late May through mid-June (Cameron 1970), and slightly later in more northern areas (Rothwell, personal observation). Second generation damage is frequently reported in the southern regions of the northeast, but this

injury is normally less severe than first generation feeding (Vittum et al. 1999, Vittum 1999).

Based on currently known information of *L. maculicollis* life cycle, this study has four objectives: 1) determine base temperature growth for *L. maculicollis* larvae to be used in a more accurate prediction model than is currently available, 2) determine optimal movement temperature for adult weevils to precisely time spring chemical applications, 3) investigate reproductive seasonality to better understand the *L. maculicollis* life cycle, and 4) conduct a preliminary study to determine environmental cues weevils used to terminate the overwintering phase.

According to Tashiro and Straub (1973), hibernation emergence commences at the time of *Forsythia* spp. full bloom. Normally, all adults will have moved onto golf course turf at the time of full bract of flowering dogwood, Cornus florida L. Because we currently lack accurate seasonal history data, golf course superintendents rely on plant phenology to apply spring insecticide applications, and most treatments are applied between one week after Forsythia spp. full bloom and dogwood full bract. Second generation applications are made based on calendar days, around 4 July. Cameron (1970) investigated L. maculicollis seasonal habits, but found no consistent pattern of cyclic behaviors to develop into a precise prediction model; both Cameron (1970) and Vittum (1980) found all life stages on any given date from late June through late August. Vittum (1980) attempted to provide a degree-day (DD) model for spring generation larvae, but due to external factors, such as variable adult emergence, lack of an exact base temperature, as well as an antiquated prediction system, she was not able to precisely predict overwintering adult emergence and peak larval damage. This model was,

however, able to forecast activity of second-generation larvae and can be used to predict optimal timing of insecticide applications targeting second generation adults. Overall, based on these initial studies, spring larval activity appeared to be more highly correlated with calendar dates than with thermal units (Vittum 1980).

The purpose of this study was to build a precise forecasting system for golf course superintendents to accurately predict L. maculicollis larval activity and their associated damage in the field. This investigation also aimed to determine when monitoring should be carried out most aggressively throughout the season. To this end, we investigated the relationship between thermal units and spring damage of L. maculicollis larvae. Although no established prediction model for forecasting peak spring larval activity is currently used, Vittum (1980) employed the base temperature 10° C and reported peak summer larval activity at 1,050 DD. The most commonly used threshold temperature for L. bonariensis is 10° C (Kelsey 1958, May 1961, Pottinger 1961, Power and Singh 1974, Goldson 1979, Goldson et al. 1982, Barker 1988) and for L. oregonensis models, most researchers use the base temperature 7° C (Simonet and Davenport 1981, Boivin 1988). We attempted to establish an accurate threshold temperature for L. maculicollis larval growth. This information can be used to determine the relationship between DD accumulations, adult emergence, and peak larval activity to accurately predict peak spring larval populations.

In addition to the traditional degree-day model, we wanted to provide superintendents with a time frame in which to apply chemical control targeting adults as these measures are the most commonly practiced to minimize spring larval damage (Tashiro and Straub 1973, Vittum 1999). Predicting peak adult spring emergence is

essential for an accurately timed spring application, as one well-timed spring adult treatment has provided adequate control for later season larval populations (Vittum and Rothwell 2000). Therefore, we need to establish an optimal temperature at which adults move in the spring in order for golf course superintendents to refine their monitoring practices and to enhance their efficacy of spring applications.

Preliminary observations of L. maculicollis seasonal habits have been made (Cameron 1970, Cameron and Johnson 1971, Vittum 1980, Vittum and Tashiro 1987), but more information on physiological development over time is needed. Vittum (1980) indicated L. maculicollis undergo a reproductive diapause given its ability to oviposit over an extended period in the spring. Sex organs of insects that undergo a reproductive diapause are undersized during the winter months, and environmental cues trigger organ growth to enable insects to mate in the spring. Insects in the state of reproductive diapause are not capable of reproducing (Goldson 1981), so in order to oviposit in the spring, sex organs must increase in size, and this growth time appears to be variable in L. maculicollis (Vittum 1980). L. oregonensis (LeConte) (Ryser 1975, Stevenson 1977, Boivin 1988, Stevenson and Boivin 1990) and L. bonariensis (Kuschel) (Goldson 1979, Goldson 1981, Barratt et al. 1995) undergo reproductive diapause in winter months. L. bonariensis undergo diapause in regions of New Zealand, even when few temperature extremes are recorded; this reproductive cessation was noted in weevil diapause in Argentina (Goldson and Emberson 1980). Weevils recently introduced to New Zealand are suspected to continue to undergo relict diapause as observed in their native South American habitat (Goldson and Emberson 1980).

Because reproductive diapause is widespread in *L. bonariensis*, even in spite of relocation, it is important to investigate the effects of this state on the closely related *L. maculicollis*. Diapause affects spring emergence and, ultimately, oviposition period and the overall seasonality of the insect. Because our aim is to control ovipositing spring females, we must determine how these weevils are affected by reproductive diapause. We performed preliminary investigations to determine if *L. maculicollis* undergoes, similar to its counterpart *L. bonariensis*, reproductive diapause by characterizing the different stages of reproductive organ development over twelve months (Goldson 1981 Methods used). Knowledge of adult oviposition period should enable turf managers to increase the effectiveness of a variety of strategies to suppress the subsequent larval population.

Because diapause influences oviposition timing, the potential factors regulating *L. maculicollis* oviposition need to be investigated. Oviposition in other *Listronotus* weevils, for example, the carrot weevil *L. oregonensis*, is influenced by temperature and photoperiod (Whitcomb 1965, Martel et al. 1976, Stevenson 1977, Simonet and Davenport 1981, Stevenson and Boivin 1990). Photoperiod alone determines the onset of oviposition in *L. bonariensis* (Goldson 1981, Barratt et al. 1995). These studies will provide a basis for investigation into the onset of oviposition in *L. maculicollis*. Vittum (1980) suggested that overwintered females begin to lay eggs in response to temperature and photoperiod cues. We also investigated whether overwintering females are inseminated or if they must locate a mate in the spring, prior to ovipositing.
Methods and Materials

Prediction Model Base Temperature: Larval Growth. Larvae were collected from Hartford Golf Club, Hartford, CT on 10 and 17 June 2001. We had intended to sample a range of instars, but many of the small larvae (1st and 2nd instars) were too difficult to see and proved impossible to collect. Thus most larvae collected were 3rd, 4th, or 5th instars. We monitored larval growth in environmental chambers at six different temperatures (°C): 7, 12, 14, 16, 18, and 24. At least 50 larvae were held at each temperature. The insects were held at a photoperiod of LD 16:8. Each larva was placed in a 40 mmdiameter, 35 mm deep plastic cup with a moistened dental wick. Every three days, we measured the widest part of the head capsule from a full, straight-on view of the epicranial sutures and dorsal mouthparts directly under the dissecting microscope lens. We scored a larva as growing if the change from the previous measurement was greater than 0.15 mm. Dead larvae were discarded. Larvae were maintained in the growth chamber until they reached the pupal or adult stage. Changes from pupa to adult were also recorded.

Because the 2001 data showed head capsule change between 12 and 14° C, we refined our methods by observing larval growth at 12, 13 and 14° C in 2002. We replicated the study mentioned above to determine a more precise temperature for larval growth. We collected 3rd, 4th, and 5th instars from Hartford Golf Club on 27 May. At least 30 larvae were individually maintained in growth chambers set at 12, 13, and 14° C.

Cups, as described above, were used for each temperature setting. Head capsules were measured as above, and pupa to adult changes were also noted.

Prediction Model: Golf Course Temperatures and Listronotus maculicollis

Sightings. Air and soil temperature data were collected daily for three years (2000, 2001, and 2002) between 0700 and 0900 at four golf courses in the northeastern United States: Sleepy Hollow Golf Club, Scaroborough, NY; Dunwoodie Golf Club, Yonkers, NY; Hartford Golf Club, Hartford, CT; and Back O' Beyond Golf Club, Danbury, CT. Air temperatures were collected from 1 March until 1 July, and soil temperature measurements began as soon as snow was removed from the designated area and continued to 1 July. Air temperatures were collected with a minimum/maximum thermometer placed at or near a site on the golf course where L. maculicollis damage occurred seasonally or using a permanently fixed weather station. If a minimum/maximum thermometer was used, it was placed in an area without full sun or full shade. Soil temperatures were taken with a standard soil thermometer inserted ca. 8 cm into the soil. Three times weekly, superintendents collected surface temperatures from weevil overwintering sites by placing a thermometer in leaf or pine litter or mossy cover of a hibernating area until the thermometer had reached equilibrium. Precipitation levels were measured daily with weather station equipment or a standard rain gauge.

Spring emergence of adult weevils was monitored during 1999-2002 with linear pitfall traps at the four golf courses. Linear pitfall traps were constructed of 1.2 m polyvinyl chloride (PVC) piping with a 5.4 cm diameter. Nine 3.2 cm-diameter holes were drilled into the length of the piping with a 7.0 mm space between the margins of

each hole. Funnel-shaped plastic cups, with bottoms removed, were inserted into the holes and secured with waterproof caulking. Traps were placed in the ground at a slight slope, with the tops of the cups flush with the surface of the turf. Of the two pipe ends, the higher one was sealed while the lower open end of the trap was attached to a 3.8 L plastic jug, which was equipped with drainage holes. Because the pitfall traps were inserted in the ground at a slight angle, they were cleared three times per week by flushing water through the top-most cup, which flushed weevils caught in the trap into the collection jug. Traps were placed in the rough-mowed turf of each golf course, less than five meters from a known *L. maculicollis* overwintering site. The traps were placed in the ground on 1 March, or soon as possible thereafter depending on snow cover.

Beginning 15 March, in each of three years (2000, 2001, and 2002), we monitored each course three times per week for the presence of adult weevils on short-mowed turfgrass: tees, greens, and fairways. Adult weevil occurrence was based on visual inspections of the turf surface as well as on vacuuming. We used a modified leaf blower (Homelite[®], Anderson, SC, d25mhv, UT08084) to remove adult weevils crawling across short-mowed turf; each golf course was randomly vacuumed for five-minute intervals in three 5 m x 5 m areas known to have had past *L. maculicollis* damage.

On 20 May 2000, we began to search for *L. maculicollis* larvae present in the golf course fairways, greens, and tees. In 2001 and 2002, our search for larvae began on 1 May. This earlier date allowed for a more accurate date of first larval presence on the golf course. We used a 5 cm-diameter turf-corer (Turf-Tec International[®], Oakland Park, FL) and collected 30 turf cores weekly from each golf course from areas with known

weevil damage. Cores were brought back to the lab, where they were hand-inspected for larvae. Number of larvae and stage of development were recorded for all dates.

Optimal Adult Movement Temperature. We performed a study to determine the optimal movement temperature for spring emerging adults. Leaf litter containing adult *L. maculicollis* was gathered from overwintering sites at Woodstock Country Club, Woodstock, VT on 16 January 2002. The air temperature at the time of collection was -2° C. Weevils were returned to the laboratory where they were extracted by floating them out of the leaf litter using lukewarm water (Vittum 1980). Captured weevils were placed in screen-topped 250 ml plastic containers and held in a cooler at 6° C.

Weevils were removed from the cooler, placed in an environmental chamber at one of six temperatures (0, 5, 10, 15, 20, 25° C), and held for 24 hours. We then removed an individual weevil and placed it onto a 1.22 m x 0.75 m table covered with white paper in a room held at 7° C, which was the coldest place available to conduct the experiment. The weevil was placed ventral-side down with its head facing east. Weevil movement was observed for five minutes and its position was noted every minute. Each movement segment was measured as straight-line distance from the weevil's previous position. Total movement was the sum of the segments.

The adult movement temperatures collected in the laboratory were correlated to adult activity in pitfall traps in the field. These traps, as described above, were placed in overwintering locales prior to weevil emergence. These traps collected first emerging weevils at the four golf courses. Using daily maximum temperature at each golf course, we correlated the first weevil collection from pitfall traps to the ambient air temperature for 1999, 2000, 2001, and 2002. Since we collected trapped weevils only once a week, we used the highest temperature from the three days prior to collection to best correlate air temperature with adult activity.

Reproductive Seasonality. To determine the reproductive seasonality of *L. maculicollis* adults, we dissected weevils for each month in 2000 and 2001. We collected weevils at least twice each month from golf course turf at Sleepy Hollow Golf Club, Scarborough, NY. During the growing season, adult weevils were collected from short-mowed turf with a modified leaf blower that performed like a vacuum, as described previously. In winter months, overwintering insects were gathered from leaf litter in known hibernation sites and were extracted from leaf litter as described above. Adults, collected during summer and winter months, were chilled to 6° C upon return to the laboratory. During all months, we collected as many weevils as possible. Collection numbers ranged from five weevils in late July to as many as 150 weevils in mid-May.

For each month, a minimum of 20 females and 20 males was dissected under a light microscope at 45X. Male weevils were dissected after death, but females were chilled and dissected alive to verify presence of actively moving sperm. Using a hot probe, individual weevils were embedded into a petri dish filled with a mixture of hardened paraffin and lamp black. Extra wax was built up around the sides of the insect so only wing covers were visible, which ensured live weevils would be immobile during dissection. Before dissection, 0.01% saline solution was poured over embedded weevils to prevent desiccation of reproductive organs. The elytra and the outer epidermis were removed from each insect to expose reproductive organs. In female weevils, length of

ovary and the width of germaria were measured. The number of eggs in the right ovariole was counted. The spermatheca was extracted, placed on a slide with distilled water, covered with a glass cover slip, and gently crushed to release the motile sperm, and we verified presence or absence of sperm under a 10X dissecting microscope. In male weevils, we measured the widths of the seminal vesicle and accessory gland as well as the diameter of the prostate gland.

Measuring Presence/Absence of Sperm in Overwintering Females. To determine whether females were inseminated prior to overwintering or if they mated upon emergence in the spring, we dissected 80 weevils in December 1999 and 2002 from three golf courses: Hartford Golf Club, Hartford, CT; Woodstock Country Club, Woodstock, VT; and Sleepy Hollow Golf Club, Scarborough, NY. Leaf litter from known *L. maculicollis* hibernation sites was gathered from each golf course and brought back to the laboratory, where the weevils were collected by flotation (Vittum 1980). Males and females were separated, and we determined presence or absence of sperm from female spermathecae by dissection methods described above.

Preliminary Investigation of Cues that Terminate Reproductive Diapause. In 2001, we conducted a preliminary experiment to determine latent cues that herald *L. maculicollis* adult emergence in the spring. We examined the influence of light and temperature on the reproductive organs of spring-emerging weevils. Weevils were collected in November from overwintering sites adjacent to golf courses, and placed in a 4° C cooler for two months. Rothwell (unpublished data) determined that adult weevils

need a minimum of two months in cold temperatures to become reproductively active. In order to determine the environmental triggers that terminate reproductive diapause, we set one growth chamber at 5° C, LD 16:8 and the other chamber at 22° C, LD 8:16. Fifty females were placed in each chamber. In separate containers, we placed 50 males in the two growth chambers. We added five males to the containers with the fifty females, and five females were placed with the male weevils to ensure proper reproductive behavior. Ten weevils were removed from each chamber every four days until 20 days had passed. Insects taken from the growth chambers were dissected and reproductive organs were measured as previously explained.

Statistical Analysis. To analyze the optimal movement of adult *L. maculicollis*, we compared the mean distance traveled by the weevils at each temperature using analysis of variance (ANOVA). Differences among these treatment means were tested using Tukey's Pairwise Comparison at $\alpha = 0.05$. The statistical package Minitab[®] (2000) was used for this analysis.

Results

Prediction Model Base Temperature: Larval Growth. In 2001, the minimum temperature at which a change in head capsule width was recorded was 14° C (Figure 4.1). Twenty-two percent of larvae were observed to have an increase in head capsule size at this temperature whereas 50% of pupae changed to adults. We found no change in larval head capsule size at 7° or 12° C, but larvae lived for up to 30 days at these temperatures. As temperature increased, we observed more changes in head capsule size.

We observed an average mortality rate of 83% in this study and attribute it to lack of food.

When we repeated the study in 2002, three of 30 weevils showed an increase in head capsule size at 13° C (Figure 4.1). As in 2001, we observed no change in head capsule width at 12° C, and pupae did not molt into adulthood at this temperature. At 14° C, 60% of pupae successfully emerged as adult weevils.

We intended to use the optimal laboratory-generated base temperature to calculate degree-days, but because growth of larvae in the laboratory may be different than the growth in the field, we entered a series of temperatures in the commonly used degree-day model equation (Elkinton 1993):

(<u>Maximum daily temperature-Minimum daily temperature</u>) - base temp = DD accumulated 2

to determine the optimal base temperature for *L. maculicollis* (Table 4.1). Arnold (1959) found that the base temperature yielding the least variation between years of a study is appropriate base temperature. None of the tested temperatures provided minimal variation between the years, but the higher temperatures were more suitable than the lower temperatures. Temperatures 13.3° C and 14.4° C showed the least variability, but even these temperatures were not optimal. Since we did observe laboratory growth, albeit minimal, at 13° C, and more larvae showed change at 14° C, it appeared that an intermediate temperature would be appropriate, and we chose 13.3° C as the base temperature for growth of *L. maculicollis* larvae. Of the temperatures tested in the DD equation for the three years the study, this temperature also seemed optimal. In addition, 13.3° C equals 56° F, which can be easily used by golf course superintendents in the field.

Prediction Model: Golf Course Temperatures and Listronotus maculicollis

Sightings. The base temperature of 13.3° C was applied to all ambient air temperature data from the four participating golf courses and compared degree-days and the first reported sightings of *L. maculicollis* larvae. The results of these calculations are listed in Table 4.2. In 2000, the degree-days for Sleepy Hollow were higher (230 DD) than the remaining golf courses. The span between degree-days calculated for the four golf courses was less variable in 2001, where first larvae were observed on the golf courses between 156 DD and 205 DD. In the final year of the study, we reported the first sighted larvae between 122 DD and 199 DD. Overall, degree-days indicated a presence of larvae on golf course turf between 150 DD and 200 DD using a threshold temperature of 13.3° C and air temperatures on 1 March.

Optimal Adult Movement Temperature. We detected a significant difference in adult movement at the six temperatures (F $_{6, 132} = 6.5$; P < 0.001, Figure 4.2). When *L*. *maculicollis* adults were held at 20°C for 24 hours, we saw a significant increase in movement with comparison of means (P < 0.05).

We compared adult laboratory movement to adult weevil activity in the field using the pitfall trap data (Table 4.3). We used the highest temperatures of three days prior to monitoring pitfall traps catches to determine at which temperatures adults were moving in the field. In the laboratory, the optimal temperature for adult *L. maculicollis* movement was 20° C, and each golf course in the three years of the study, we captured the first emerging adults within 4.5° C of our determined laboratory movement temperature. The one exception was Dunwoodie Golf Club, where first adults were observed at 26.1° C in 2001.

Reproductive Seasonality. Female reproductive organ size increased during the growing season and decreased during colder months (Figure 4.3). A significant detail to note is the correlation of ovarian growth at the beginning of May of each year and the decline in ovary length between September and October. In 2000, ovary length increased in May, June, July, August, and September, and we observed similar lengths in 2001. We noted a decrease in size in mid-summer, around July, which suggests two generations. Germaria widths have a similar pattern to that of ovarian development, but they were less pronounced (Figure 4.3 b).

Detection of eggs in the right calyx is noted in Figure 4.4. In both years, we found an increased number of eggs in May at the same time that ovary length was at its peak. There were no eggs reported in the winter months. The presence of sperm in the spermathecae also changed with the season (Figure 4.5). During both seasons, insemination peaked in May, and we detected sperm in 100% of dissected females. When we performed dissections in August, we found 100% of females were inseminated in 2000 and 79% were inseminated in 2001. Females dissected in the winter months had low insemination levels.

L. maculicollis males revealed similar patterns in seasonal variation to that of females (Figure 4.6). The three organs measured (seminal vesicle, prostate gland, and accessory gland) increased in size in the spring, beginning in March/April. However, a

decrease in organ size in July was noted in both years, followed by an increase in August, and finally a steady decrease through the fall.

Measuring Presence/Absence of Sperm in Overwintering Females. Percent female insemination was monitored at three different golf courses in 1999 and showed substantial geographical variation (Figure 4.7). About 11% and 13% of females collected from New York and Vermont, respectively, were inseminated prior to overwintering. However, Connecticut females demonstrated a higher level of insemination; 55% had sperm present in their spermathecas prior to hibernation.

Overwintering females collected and dissected in 2002 showed similar insemination levels. The rates were 40%, 7%, and 14% for Connecticut, New York, and Vermont, respectively.

Preliminary Investigation of Cues that Terminate Reproductive Diapause. The preliminary examination of reproductive diapause provides initial information as to the cues weevils use to emerge from hibernation in the spring. We observed a trend of increased organ growth with warmer temperatures and shorter daylengths. Increases in female ovarian development were detected in weevils placed in LD 8:16 and 22° C on the 20th day of the experiment even though the light regime reflected mid-winter conditions, whereas no changes were detected in females at LD 16:8 and 7° C (Figure 4.8). Germaria widths increased in females placed at 22° C and LD 8:16 compared to those in 7° C and LD 16:8 (Figure 4.8). Females placed in LD 8:16 at 22° C showed a trend of more eggs than females held at LD 16:8, 7° C, and more females were inseminated in the warmer

temperatures with shorter daylengths (figure 4.9). Males held in LD 8:16, 22° C also showed trends of larger organ size than males held in LD 16:8, 7° C (Figure 4.10).

Discussion

From this study, we have determined the base temperature for larval growth to be 13.3° C. This temperature can be placed in a degree-day equation using collections of ambient air temperatures, and superintendents can use the accumulations to predict *L. maculicollis* larvae on golf course turf. We also determined the optimal adult movement temperature to be 20° C, which also provides golf course managers with improved management strategies for *L. maculicollis*. Reproductive seasonality has also been investigated, and these dissections have determined *L. maculicollis* undergoes reproductive diapause and has two generations per season.

Our results enable us to provide golf course superintendents with improved recommendations for updating management tactics for *L. maculicollis* on golf course turf. On 1 March, superintendents should begin recording average daily ambient air temperatures and enter them into the degree-day model equation using the base temperature 13.3° C (56° F). This temperature is higher than the temperature used in the previously developed degree-day model, 10° C (Vittum 1980). Degree-day accumulations should be calculated, and golf course managers should expect to see the first small *L. maculicollis* larvae around 150 DD-200 DD (13.3° C). Although this method will not pinpoint the presence of larvae on golf course turf, this span of degree-days will provide golf course superintendents with a more accurate timing method to begin monitoring for larval activity. Currently, most superintendents use damage as an

indicator of larval presence, and often, by the time damaged turf is observed, control methods are not effective against the large population of larvae.

The discrepancies with our degree-day data may represent inaccuracies with timing of sampling, microclimates on the golf course, or placement of the temperature collection device. We sampled too late in 2000, and we did not discover larvae until damage was already reported at Sleepy Hollow Golf Course; our delay in sampling date undoubtedly contributed to the markedly high DD accumulated on that course. This golf course has only one weather station to collect air temperatures for the entire course, and reports have shown a ten-day discrepancy between differing locations across the golf course (T. Leahy, personal communication). Therefore, air temperatures collected at one location, often near maintenance facilities, may not accurately represent the timing of larval activity at a different site on the golf course. In addition, one year we detected larvae at 122 DD at Back O' Beyond Golf Course, which was earlier than the other collection sites. The site where we sampled often experiences higher temperatures than other areas on the golf course (M. Maffaei, personal communication), and these warmer temperatures may result in early oviposition of emerging adult females. Therefore, intensive sampling must be performed to accurately determine presence of L. maculicollis larvae. Thorough sampling will eliminate some inconsistencies.

In addition to larval monitoring, superintendents must consider adult emergence in the spring, since this stage of the insect is targeted for control. Although adult weevil movement occurs at lower temperatures, we showed that 20° C is the peak movement temperature in the laboratory. Because there is a relationship between this temperature and the pitfall catches in the field, golf course managers can use it in conjunction with the

previously determined phenological cues associated with adult spring emergence (*Forsythia* spp. full bloom and dogwood full bract) to accurately schedule chemical applications. Potentially, superintendents can apply one well-timed treatment for adults than the multiple spray approach currently used (Vittum 1999). Vittum (1999) demonstrated that spring applications of insecticides provide adequate control from larval damage in most situations.

This research also offers insight into the natural history of *L. maculicollis*. Female weevils show no distinct pattern of insemination prior to overwintering, but in most locations female weevils are unlikely to be inseminated before they enter hibernation. This insect also appears to undergo a reproductive diapause during the winter months as demonstrated by the small size of reproductive organs in the winter months. Changes in organ size of *L. maculicollis* are similar to those documented in *L. bonariensis*, and Goldson (1980) confirmed that these weevils undergo a state of reproductive diapause. Studies investigating the hormones associated with reproductive diapause as well as body fat content would be logical subsequent steps to determine the onset as well as the termination of this state. Our initial results suggest that the termination of reproductive diapause was more likely by warmer temperatures and shorter daylengths than colder temperatures and longer daylengths, and it would be informative to expand this study to tease apart the effects of temperature and daylength.

Our reproductive seasonality studies have verified *L. maculicollis* have two generations per season based on changes in reproductive organ size of male and female weevils. Throughout the 12 months, both sexes showed parallel patterns of increases and decreased in organ size. May and August are the primary oviposition months because

both number of eggs and insemination levels are at their peak. Additionally, male and female reproductive organs are larger in the warm part of the season than those recorded in the winter months, which is indicative of sexual activity during the spring and summer. Golf course managers can use this seasonal history to help predict weevil life cycle on golf course turf in order to adequately manage the two generations per season.

Overall, this study has provided a more comprehensive description of the life history of a recurring golf course pest. This information will aid golf course superintendents to accurately monitor and subsequently control these insects. Managers previously relied on calendar dates to apply chemical treatments, but with this current information, they can use the adult optimal movement temperature to target emerging spring adults. Well-timed applications will increase efficacy, which in turn, should reduce the number of chemical treatments needed on the golf course. In addition, we constructed a simple prediction system that will estimate the first generation larvae. Lastly, our research describes weevil reproductive activity throughout the growing season, and this information will help managers make better overall decisions when managing *L. maculicollis* in the field.

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Figure 4.1. Percent of *Listronotus maculicollis* larvae that showed head capsule size change when held in growth chambers at a series of temperatures. These temperatures were determined for the threshold temperature for larval growth to be used in a degree-day equation. At least 50 larvae were used at each temperature in A) 2001 and 30 in B) 2002.



Figure 4.2. Mean adult *Listronotus maculicollis* movement at seven different temperatures (° C) \pm SE. Movements were measured for five minutes in one-minute increments. The total distance traveled is summed for all movement within the time period. An asterisk (*) indicates differences in the distances traveled by adults (F _{6, 132} = 6.5, P < 0.001), and Tukey's Pairwise Comparison showed weevils to move more significantly at 20° C than other temperatures.



B



Figure 4.3. Listronotus maculicollis ovary lengths and germaria widths (mm) \pm SE recorded for 12 months in 2000 and 2001. Measurements are means of organ size from a minimum of 20 weevils collected twice per month to provide one mean per month.



Figure 4.4. Mean number of *Listronotus maculicollis* eggs \pm SE recorded for 12 months in 2000 and 2001 (N = 20). Means are derived from eggs counted in the right calyx. At least 20 weevils were dissected each month.



Figure 4.5. Percent of adult female *Listronotus maculicollis* that were inseminated for 12 months in 2000 and 2001 from Sleepy Hollow Golf Club, Scarborough, NY. At least 20 weevils were dissected each month.



Figure 4.6. Male *Listronotus maculicollis* reproductive organ measurements (mm) \pm SE recorded for 12 months in 2000 and 2001. Measurements are means of seminal vesicle widths, prostate gland diameters, and accessory gland widths. At least 20 weevils were dissected each month.





Figure 4.7. Percent of inseminated overwintering female *Listronotus maculicollis* in 1999 and 2002. At least 80 females were dissected in each year at each site.



B

A



Figure 4.8. Measurements of female *Listronotus maculicollis* a) mean ovary length \pm SE and b) mean germaria width \pm SE under two conditions: 16:8 LD, 7° C and 8:16 LD, 25° C. All measurements are means of a minimum of 10 dissected female weevils.



B



Figure 4.9. Measurements of female *Listronotus maculicollis* a) eggs \pm SE and b) percent insemination under two conditions: LD 16:8, 7° C and LD 8:16, 22° C. All measurements are means of a minimum of 10 dissected female weevils.



Figure 4.10. Measurements of male *Listronotus maculicollis* a) seminal vesicle \pm SE and b) prostate gland \pm SE and c) accessory gland \pm SE under two conditions: LD 16:8, 7° C and LD 8:16, 22° C. All measurements are means of a minimum of 10 dissected males.

Table 4.1. Comparison of temperatures to be used in a degree-day equation to predict first sighting of *Listronotus maculicollis* larvae. The air temperatures used in the equation were collected from Hartford Golf Club, Hartford, CT; Back O' Beyond Golf Club, Brewster, NY; Sleepy Hollow Golf Club, Scarborough, NY; and Dunwoodie Golf Club, Yonkers, NY. The degree days in the table were calculated from five potential base temperatures, and the temperature that produces the least variation between years should be used to in the degree day equation.

		Ba	se temperature	(° C)	
Year	11.1	12.2	13.3	13.9	14.4
2000	363.1	280	173.5	182.5	152.6
2001	277.8	228.3	183.9	164.5	147.3
2002	245.6	198.6	160.9	144	129
± SE	20.4	15.5	9.0	9.9	8.6

Table 4.2. Degree-days for first sighted *Listronotus maculicollis* larvae at Hartford Golf Club, Hartford, CT; Back O' Beyond Golf Club, Brewster, NY; Sleepy Hollow Golf Club, Scarborough, NY; and Dunwoodie Golf Club, Yonkers, NY. Degree-days were calculated using the equation (average air temperature) – (base temperature). The base temperature used to calculate degree-days in the table was 13.3° C.

Golf course		Degree-Days	
	2000	2001	2002
Hartford	174	170.5	149
BOB	148	156	122
SH	230	204	199
Dunwoodie	169	205	173.5

Table 4.3. Dates of first sighted adult *Listronotus maculicollis* on four different golf courses for 1999, 2000, 2001, and 2002. Highest recorded temperature (°C) three days prior to adult collection was used.

1999				
Golf Course	Date	Number Adults	Max. Temp	
Hartford	2 April	17	23.8	
BOB	2 April	63	19.4	
Sleepy H.	19 April	17	19.4	
Dunwoodie	20 April	2	17.8	

2000___

Golf Course	Date	Number Adults	Max. Temp
Hartford	25 March	3	20
BOB	1 April	1	16.7
Sleepy H.	20 March	20	17.2
Dunwoodie	7 April	2	20

2001

Golf Course	Date	Number Adults	<u>Max. Temp</u>
Hartford	5 April	3	15.5
BOB	3 March	2	16.7
Sleepy H.	13 April	15	20.7
Dunwoodie	12 April	35	26.1

Golf Course	Date	Number Adults	<u>Max. Temp</u>
Hartford	5 April	15	21.3
BOB	2 April	6	20.2
Sleepy H.	19 April	22	18.7
Dunwoodie	20 April	13	19.8

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