EFFECTS OF MOWING ON ANNUAL BLUEGRASS WEEVIL,  
LISTRONOTUS MACULICOLLIS KIRBY (COLEOPTERA: CURCULIONIDAE)  
ADULT BEHAVIOR AND LARVAL DEVELOPMENT  
IN GOLF COURSE PUTTING GREENS  

A Thesis in  
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by  
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Abstract

The annual bluegrass weevil (*Listronotus maculicollis* Kirby) is the most destructive insect pest of low-mown golf course turf in the northeastern and mid-Atlantic United States, and southeastern Canada. Golf course superintendents rely heavily on chemical controls, particularly on high-valued turf areas such as fairways, tees, greens, and their immediate surrounds (collars). These areas, particularly putting greens, are of the highest value to the course and the game of golf. Therefore, multiple insecticide applications, targeting both adults and larvae, are made throughout the year, often using the same insecticide classes. The overuse of insecticides, particularly the pyrethroids, has resulted in an increase in insecticide-resistant populations, and a dire need to develop alternative control strategies. I investigated the effect that cultural practices have on *L. maculicollis* survival, behavior, and development in golf course putting greens to determine if populations may be reduced in these areas without synthetic insecticides and to develop Best Management Practices (BMPs) for putting greens.

We know very little about the effects that turfgrass cultural practices (e.g. mowing, fertilization, irrigation) have on *L. maculicollis* population development and damage expression, particularly in golf course putting greens. Larval feeding damage is rarely reported in these areas, but is commonly observed on the adjacent short-mown turf (e.g. collars, fairways). Putting greens receive the highest input of insecticides and the most frequent and intense cultural inputs. Damage is frequently observed on collars, which receive all the same insecticide applications and cultural practices as the putting surface, with the exception of mowing height. This may suggest that adult survival and/or larval development is affected by mowing height. I found that mowing height had a significant effect on adult survival and behavior. Increasing numbers of adults were removed with decreasing mowing heights.
Surprisingly, most adults were removed alive, suggesting there is a potential for moving weevils around the golf course and creating new infestations in close proximity to where clippings are disposed. Additionally, adults deviated from their normal oviposition behavior in green’s height turf, choosing to oviposit outside of the turfgrass plant rather than within the stem of the plant. Neither larval abundance nor fitness was affected by mowing height-of-cut treatment.

I examined the effect that time-of-day (TOD) and temperature had on adult canopy activity to determine when *L. maculicollis* are most susceptible to removal by mowing. TOD had no influence on *L. maculicollis* adult activity in controlled lab experiments when temperature was held constant. However, temperature appeared to influence vertical movement within the canopy. Canopy activity was significantly reduced as temperatures decreased below 10°C. Temperature influenced canopy activity in field observations more than TOD. However, adults were most active during daylight hours, with very little activity at night. Adult activity peaked when day time temperatures were between 14 and 18°C, with reduced activity on either side of this range.

The findings of this thesis suggest that *L. maculicollis* has the potential to develop to damaging stages in turf maintained at putting green heights throughout the region. However, the act of mowing, especially at very low heights may affect population development and thus reduce the frequency of insecticide applications. The fate of loose eggs remains unknown, but the vulnerability of loose eggs may be a contributing factor to the lack of damage observed in putting greens. Finally, superintendents must be aware of how clippings are disposed of since most adults survived the act of mowing and may potentially re-infest turf neighboring disposal areas.
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Chapter 1: Review of the literature

Introduction

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is a potentially devastating turfgrass insect pest of golf course and tennis court turf. Reports of damage originated in the New York City (NYC) metropolitan area, but have continually spread from this epicenter to now include the northeastern and mid-Atlantic United States, and parts of the southeastern provinces of Canada (Vittum et al. 1999; Simard et al. 2007). Damage from crown-feeding larvae is generally observed in spring, though may re-occur throughout the entire growing season. Golf course superintendents often make several insecticide applications throughout the growing season to avoid damage (Cowles et al. 2008). Superintendents often make between 6 and 10 insecticide applications per year to keep larval densities below damaging thresholds (McGraw and Koppenhöfer 2017). The over-use of synthetic insecticides, particularly the pyrethroids, has resulted in a decrease in the susceptibility of *L. maculicollis* to several insecticide classes (Cowles et al. 2008; Ramoutar et al. 2009). The increasing prevalence of insecticide-resistant populations has resulted in a dire need for the development of alternative control strategies.

Little is known about how common golf course cultural practices (e.g. mowing, fertilization, irrigation) affect *L. maculicollis* population dynamics, especially on putting greens. Previous investigations have examined *L. maculicollis* population dynamics in lower valued turf (e.g. fairways, tees) maintained with less intense cultural practices than performed on putting greens. The black cutworm (*Agrotis ipsilon* Hufnagel), another turfgrass insect pest that lays eggs in putting surfaces, have been effectively controlled by mechanical removal of eggs with mowers on golf course putting greens (Williamson and Potter 1997). White grub densities may not be directly impacted by cultural practices, but damage severity may be lessened by altering nitrogen fertility, height-of-cut, and irrigation in golf course rough-mown turf (Potter et al. 1996). There is potential for similar tactics to be developed to aid
in the control of *L. maculicollis*. This review of the literature serves as an introduction to the insect and summary of recent research investigating the effects that golf course cultural practices have on its population dynamics and turfgrass damage.

**Taxonomy**

*Listrionotus maculicollis* is in the order Coleoptera, the suborder Polyphaga, superfamily Curculinoidea, family Curculionidae, and subfamily Cylindrorhininae (O’Brien 1979, 1981). The genus *Listronotus* has undergone significant revision, which resulted in five species previously belonging to the genus *Listronotus* to be reclassified in the genus *Hyperodes*. Currently, there are known to be 81 *Hyperodes* or *Listronotus* species listed in North America, north of Mexico (Arnett 2002). Species of *Listronotus* can be distinguished from *Hyperodes* by the second segment of the funiculus (the segment of the antennae between the club and the base of the antennae). The second segment of the funiculus of *Listronotus* spp. is much longer than the first. Another difference between *Hyperodes* and *Listronotus* is that adult *Hyperodes* tend to be significantly smaller than members of the *Listronotus* species (Kissinger 1964).

*Listronotus maculicollis* was originally categorized as *Hyperodes* sp. near *anthracinus* (Warner 1965). Samples collected from damaged golf course fairways in Long Island, New York and Pennsylvania in 1957, and 1961 contained an insect that was originally classified as *Hyperodes anthracinus* (Dietz) (Warner 1965). The same specimens from Long Island were examined by H. Dietrich and identified the specimens to be *H. maculicollis* (Kirby), a species that has been identified in all 50 states. The species was then placed between *anthracinus* and *maculicollis* due to its morphology, and designated as *Hyperodes* sp. near *anthracinus* (Dietz) (Cameron and Johnson 1971). Eventually Warner classified the insect as *Listronotus maculicollis* (Vittum et al. 1999).
There have been several common names used to describe *L. maculicollis*. The common name “Hyperodes weevil” is often used to describe the insect because of its prior classification as a *Hyperodes* spp. (Cameron and Johnson 1971, Vittum 1980). The name “annual bluegrass weevil” is the most frequently used since most damage occurs to annual bluegrass (*Poa annua* L.) within mixed turfgrass stands. However, some feel this may not be an appropriate common name for the insect because damage is often reported on *Agrostis stolonifera* L. (McGraw and Koppenhöfer 2010, Kostromytska and Koppenhöfer 2014, McGraw and Koppenhöfer 2015).

**Distribution**

*Listronotus maculicollis* is believed to be native to North America, though is currently only known to be a pest of low-mown turfgrasses (< 1.25 cm) found on golf courses and grass tennis courts in the eastern United States and southeastern Canada (Vittum et al. 1999, Simard et al. 2007). It is unknown how long *L. maculicollis* has been damaging golf course turf, but in the early 1930s reports of “spring die-out” were often made in late-May to mid-June on Long Island, New York and southeastern Connecticut (Cameron and Johnson 1971). It is possible that these reports were actually unknown cases of *L. maculicollis* damage (Vittum 1980). By 1965, several more golf course superintendents on the north shore of Long Island observed *L. maculicollis* damage (Cameron and Johnson 1971). Damage was also reported by several courses in Westchester Co., and in Ithaca, NY during this time. By the 1980s, *L. maculicollis* was reported damaging turf throughout much of New York State, most of southern New England, New Jersey, and southeastern Pennsylvania (McGraw and Koppenhöfer 2007).

Damaging populations of *L. maculicollis* have been spreading outward from the area of initial detection ever since it was discovered in the mid-1900s (Simard et al. 2007). Today, the greatest population densities continue to be found on golf courses located within the metropolitan New York City area, including parts of Long Island, Westchester county, northern New Jersey, and southern
Connecticut (Vittum et al. 1999; Simard et al. 2007). Damage has been reported as far north as Québec, south into the western mountains of North Carolina, and as far west as Ohio (Simard et al. 2007). As the insect continues to spread, it becomes problematic for superintendents who are faced with managing a pest that they have never dealt with before.

Seasonal development and life stages

Adult *L. maculicollis* will overwinter in wood lines, thick leaf litter, tall grasses and other protected areas directly surrounding playing surfaces (Vittum and Tashiro 1987, Diaz and Peck 2007). As spring temperatures rise, adults begin to migrate by walking toward short-mown turf. Sometime after *L. maculicollis* adults have emerged on playing surfaces, they will mate, and females will oviposit into low-mown turfgrass plants such as those typically found on tees, fairways and green collars (McGraw and Koppenhöfer 2007). When a suitable host plant has been located, the female will chew a notch in the plant and deposit eggs underneath the leaf sheath or inside the stem (Cameron and Johnson 1971). After the egg has hatched, the first-instar-larvae will feed internally on the stem of the plant until it has developed into a third-instar-larva. After completion of the third instar, the larvae will exit the stem of the plant and feed externally on the crown, or the actively growing portion of the turfgrass plant throughout the duration of the fourth and fifth-instar. The larval stages appear nearly identical at each instar, with only slight increases in size between stages (Vittum 1980). Larvae are generally crescent shaped, and wider in the middle than they are at either end. They range in lengths of 1 mm at the first instar to 4.5 mm at the fifth instar (Cameron and Johnson 1971).

After the fifth instar, the larva moves into the soil where it will begin its transformation into a pupa (Vittum 1980). The pupa is compact, creamy white, and diamond shaped. Unlike the adult, the pupa is relatively immobile, and will remain in the same location until it reaches adulthood. After pupal eclosion, the teneral (or callow) adult will emerge from the soil.
The teneral adult appears reddish to brown colored. After several days, the exoskeleton will harden, and darken to a grey-black color (Cameron and Johnson 1971). Adults possess fine hairs and yellow-brown scales that can give the elytra a slight mottled appearance (Cameron and Johnson 1971). As the adult ages, the scales wear off, giving the insect a jet-black appearance. There have been reports of as many as three generations of *L. maculicollis* in one growing season in the NYC metropolitan area (Díaz et al. 2008), although population studies have not been conducted for the southern range (e.g. MD, VA, NC).

**Host Plant Use**

*Lisronotus maculicollis* is most often found damaging low-cut *P. annua* on golf course collars, tees, and fairways. Both the adults and larvae feed on low-mown turfgrass plants, but it is the larval stage that can cause unsightly, and often irreparable damage (Vittum 1980). Adult *L. maculicollis* feed on the leaf blade of the plant, leaving small, superficial notches. For many years, it was believed that *L. maculicollis* fed solely on *P. annua*. There are two prominent subspecies of *P. annua* found on golf courses. *Poa annua annua* is a winter annual, germinating from seed in the fall and completing its lifecycle in the following summer (Vargas and Turgeon 2004). *Poa annua annua* is a bunch type grower most common on golf course fairways and tees, which can stand out from other turfgrass species and cultivars found on golf courses in the northeast due to its light green, patchy appearances. Damage from *L. maculicollis* larval feeding in fairways is often observed to be in this same “patchy” appearance that coincides with the spatial distribution of *P. annua annua* within the turf stand.

*Poa annua reptans* is a subspecies that is perennial in nature, with a prostrate, dwarfed morphology (Vargas and Turgeon 2004). This morphology, which is very different from that of *P. annua annua* allows *P. annua reptans* to persist under the intense management practices performed on golf course putting greens (low mowing heights, high fertility, and high irrigation volumes). Currently, there
have been no studies conducted on *L. maculicollis* population dynamics conducted on *P. annua reptans*. This is due to an inability to destructively sample from golf course putting greens. *Listronotus maculicollis* may behave much differently in *P. annua reptans* than in *P. annua annua*.

Turfgrass that is most susceptible to *L. maculicollis* damage is generally mowed between 4.4 - 12.5 mm and is typically found on golf course tees, fairways, and collars. Damage is rarely reported on golf course greens which are managed at a much lower height-of-cut (range = 2.3 – 4.4 mm) than tees, fairways, and collars (McGraw and Koppenhöfer 2017). Damage is also rarely observed in higher heights-of-cut (> 12.5 mm), though the species of turfgrasses typically found in these areas (e.g. *Poa pratensis* L., *Lolium perenne* L., *Festuca arundinacea* L.) differ from what is found at lower heights of cut (Rothwell 2003). Adult *L. maculicollis* will migrate by potentially walking hundreds of meters through golf course rough to oviposit in turfgrasses that are managed at the preferred height-of-cut (Diaz and Peck 2007). The predominant turfgrass species found in these areas in cool-season turf growing regions are *P. annua*, and *Agrostis spp.* (most commonly *Agrostis stolonifera* L.) (Lyman et al. 2007).

Although it was generally believed that *P. annua* was the preferred turfgrass species of *L. maculicollis*, recent studies show that height-of-cut may play a greater role in the choice of host plant acceptance. No significant spatial associations were detected between the distribution of *P. annua* and either *L. maculicollis* adults or larvae in a mixed *P. annua* - *A. stolonifera* fairway study in New Jersey (McGraw and Koppenhöfer 2015). The spatial distribution of *L. maculicollis* stages in these areas showed a bias towards fairway edges, independent of turfgrass species. This suggests is that *L. maculicollis* adults walk through fairway edges, and place eggs in low-mown hosts, independent of turfgrass species.

*Listronotus maculicollis* damage has been observed on turfgrass species other than *P. annua* (Rothwell 2003; Kostromytska and Koppenhöfer 2014; Kostromytska and Koppenhöfer 2016). This is
particularly true in the southern range of its distribution (e.g. southeastern Pennsylvania, Maryland, Delaware, and Virginia). Kostromytska and Koppenhöfer (2014) demonstrated that *L. maculicollis* are capable of oviposition in several different *Agrostis* spp. and cultivars. The study also showed that all life stages are capable of feeding and developing on *Agrostis* spp. However, larval weight was reduced and developmental time increased significantly when developing in these species compared to *P. annua*. In a separate study, larval fitness was increased when *L. maculicollis* developed within *P. annua* compared to *A. stolonifera*, though adults did not show a significant ovipositional preference for turfgrass species (Rothwell 2003).

The perception that *L. maculicollis* preferentially attacks *P. annua* may have arisen due to differences in plant tolerance (Kostromytska and Koppenhöfer 2014; Kostromytska and Koppenhöfer 2016). Models based on turfgrass damage as a function of larval density and turfgrass species suggest that as little as 10 larvae per 0.09 m² can cause damage in *P. annua*, whereas more than 150 would be required in *A. stolonifera* before damage is observed (McGraw and Koppenhöfer 2010). It is possible that *L. maculicollis* infest *A. stolonifera* and *P. annua* in equal densities, but damage is only seen in *A. stolonifera* when densities become exceedingly high.

**L. maculicollis** management tactics

Golf course superintendents rely almost entirely on synthetic-chemical insecticides to manage *L. maculicollis* populations (McGraw and Koppenhöfer 2017). Preventive applications are generally made in the spring, targeting adults moving between overwintering sites and short-mown playing surfaces. However, superintendents may make multiple applications throughout the growing season to prevent larval damage (McGraw and Koppenhöfer 2007; Cowles 2008). Curative applications targeting larvae are also an option in situations where the adulticide did not provide adequate suppression, as is often
the case with the high density populations (McGraw and Koppenhöfer 2010). There are currently no other cost-effective methods available for controlling *L. maculicollis* (Diaz and Peck 2007).

Prior to the early 2000s, superintendents reported that one pyrethroid application, made to coincide with peak *L. maculicollis* density on short-mown turf and before females laid eggs, would provide effective control for the entire season (Ramoutar et al. 2009). However, many superintendents would also make multiple pyrethroid applications prior to peak adult density, and continue to do so throughout the season, to ensure that *L. maculicollis* populations would remain below damaging thresholds (< 10% turf loss) (Cowles et al. 2008; McGraw and Koppenhöfer 2009). The sole reliance and/or overuse of pyrethroids has led to a decrease in susceptibility of populations to this class of insecticides (Ramoutar and Cowles 2009), as well as other unrelated insecticides (Koppenhöfer et al. 2012). The development of pyrethroid- and multiple-resistant populations has created a dire need for alternative controls. Unfortunately, alternatives to synthetic insecticides are not cost-effective. Studies have shown that entomopathogenic nematodes can significantly reduce larval densities, but control is inconsistent at high population densities (McGraw and Koppenhöfer 2008; McGraw and Koppenhöfer 2009; McGraw et al. 2010). The effects of golf course cultural practices on *L. maculicollis* remain relatively unstudied, and may provide superintendents with an alternative to insecticides, or at the very least, a reduction in the frequency or quantity applied.

Mowing is the cultural practice that occurs most frequently on golf course putting greens, and therefore may influence *L. maculicollis* population dynamics. Black cutworm (BCW), *Agrotis ipsilon* Hufnagel, is also a turfgrass insect pest that is found on putting greens. Regular mowing may remove as much as 90% of BCW eggs (Williamson and Potter 1997). Adult BCW moths will place eggs singly on the distal end of the leaf blade. Mowing removes the egg with the clipped leaf blade. Williamson and Potter (1997) found that as many as 50% of these eggs will survive the mowing process and can
potentially re-infest greens, tees, and/or fairways upon hatching. Research in this area has highlighted the need to manage grass clippings to ensure that clippings are placed far away from high-valued turfgrass areas. *Listronotus maculicollis*, unlike BCW, deposits its eggs in the stem of the plant. It is currently unknown if eggs are also removed with some of the plant material. It is more likely that adults foraging on the top of the canopy may be prone to removal through mowing.

**Effects of mowing**

Mowing may be the most important turfgrass cultural practice for putting greens, and is a practice that occurs almost daily on golf courses throughout the growing season. Most golf course superintendents manage tees (9.50-12.5 mm), fairways (9.50-12.5 mm), greens collars (9.50-12.5 mm), and greens (2.50-3.80 mm) below 12.5 mm. Damage caused by *L. maculicollis* is rarely observed within the interior of the putting green (McGraw and Koppenhöfer 2017), whereas damage is frequently observed on areas of higher height-of-cut such as fairways, tees, and collars or the area of turf immediately bordering putting green. One hypothesis for lack of damage on green surfaces is that adults are either removed or killed by mowers. Another theory is that the turfgrass plants in putting green turf are too short to support larval development. Understanding the mechanisms behind the phenomenon may lead to strategies to reduce *L. maculicollis* survival in these areas or at the very least, determine the probability of damage occurring on the greens and ultimately, the usefulness of insecticide applications.

The effect of mowing height on *L. maculicollis* oviposition behavior and larval development remains unstudied in putting greens. Rothwell (2003) examined the effects that mowing height has on *L. maculicollis* ovipositional preference and ability, in higher height-of-cut areas. Significantly more *L. maculicollis* eggs were found in *P. annua* mowed at tees, greens collars, fairway height-of-cut (12.7 mm) than were found in the plots mowed at rough height-of-cut (38.1mm). Almost no eggs were found in
the rough height-of-cut, leading the author to conclude that *L. maculicollis* prefer to oviposit in short turfgrass.

**Diel activity of *L. maculicollis***

Turfgrass cultural practices and insecticide applications are most commonly applied when it is convenient for the golf course maintenance staff. The timings of these practices may not coincide with increased adult *L. maculicollis* canopy activity, which may limit the effectiveness of using cultural practices as a control for adult *L. maculicollis*. A clear understanding of *L. maculicollis* diel activity, or patterns of activity throughout the day, could help determine periods when adults are most active and therefore may be affected by cultural practices, such as mowing. Identifying a time when adults are most active may also improve contact insecticide efficiency, resulting in decreased application frequency and rate.

Insects are generally most active during periods of low light, or dark periods (Danilevsky et al., 1970; Greiner 2006). Animals may differ in diel patterns for various reasons, including avoiding predators (Harmsen et al., 2011), improving individual mating success (Shi et al. 2003; Zavodska et al. 2012), or improving foraging (Cerezke 1994; Duan et al. 1996; Fedderwitz et al. 2014). Many Curculionidae species are nocturnal and show greatest activity during periods of darkness (Huang and Buss 2009). The pine weevil (*Hylobius abietis*), shows greater nighttime or “dark period” activity (Fedderwitz et al. 2014). However, they most actively feed in the hours after the end of a “dark period.” Another example of nocturnal Curculionidae species include the Warren rootcollar weevil (*Hylobius warreni*), which most actively feed at night (Cerezke, 1994). In addition, *Hylobius xiaoi* have been shown to climb trees at dusk and feed through the night, returning to the ground to rest in the leaf litter at dawn (Wen et al., 2004). Although most weevil species show increased activity during periods of dim light, there are several examples of those which show diurnal behavior (Solbreck and Gyldberg 1979;
McIntosh et al. 1996). However, this behavior may be influenced more by environmental conditions (e.g. air temperature, wind speed), rather than photoperiod.

Temperature strongly influences the diel patterns of cold-blooded organisms (Ashwood-Smith 1970). Cold-blooded organisms rely on the temperature of their surroundings to regulate their body temperatures. If environmental conditions result in a body temperature that is too cold, or too warm, the cold-blooded organism cannot perform basic biological tasks properly (e.g. metabolism, locomotion, digestion) (Ashwood-Smith 1970). Insects are a cold-blooded organism and have been observed demonstrating differences in diel patterns in relation to air temperature (Willmer 1983). The apple blossom weevil (Anthonomus pomorum L.) exhibits a predominantly nocturnal activity in spring (Duan et al., 1996). However, in the summer as day time temperatures increased from 5° C to 20° C, diel patterns became less distinctive and total crawling, mating, feeding and flight activity increased without relation to time-of-day. The Argentine stem weevil (L. bonariensis), is most active at night during the warmer summer months (Barker and Pottinger 1986), though temperature also strongly influenced adult activity. In the winter, adults were most active during warm, sunny daylight periods, as nighttime temperatures were below the lower threshold for activity. Peak activity increased with temperature increases from 5° C to 45° C in the laboratory. This indicates that L. bonariensis diel patterns are more strongly influenced by temperature than time-of-day. Rothwell (2003) assessed the effect of temperature on L. maculicollis movement in the laboratory. Adult movement, as measured by distance traveled from a central point over a 5-minute period, was greatest at 20° C. No significant differences were detected in adult movement among any temperature treatments above or below 20°C. However, these studies were only conducted in the laboratory under lighted conditions and do not imply the effect of time-of-day on adult activity.
Purpose of research

The purpose of this research is to determine the effects that mowing height has on *L. maculicollis* adult behavior and oviposition, as well as larval growth and development. Understanding the interaction between *L. maculicollis*, host plants, and management practices is essential for understanding the probability for populations to outbreak, developing alternative control strategies, and reducing unnecessary insecticide applications. I examined *L. maculicollis* ovipositional preferences, growth and development at different putting green mowing heights. I also examined *L. maculicollis* diel activity over 24 hr. periods in spring to identify a time and/or temperature range when *L. maculicollis* are most active on the turfgrass canopy and thus susceptible to mechanical removal. With the information obtained from this study, I hope to provide insights into *L. maculicollis* behavior on golf course putting greens and manage the pest in a more sustainable manner.
Works Cited


Rothwell, N. L. 2003. Investigation into *Listronotus maculicollis* (Coleoptera: Curculionidae), a pest of highly maintained turfgrass. PhD Dissertation, University of Massachusetts, Amherst, MA, USA.


Chapter 2: Effects mowing on *Listronotus maculicollis* survival, oviposition behavior, and larval development

Abstract

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is a highly destructive pest of golf course turfgrass in the northeastern and mid-Atlantic United States, and eastern Canada. Damage caused by larval feeding occurs to short-mown playing surfaces such as tee boxes, fairways, putting green surrounds (aprons and collars). However, damage to putting greens (the lowest mown surfaces) is rarely observed. I investigated the role that mowing has on *L. maculicollis* survival, oviposition behavior, and larval development in laboratory and field experiments. Greenhouse studies indicated that significant numbers of adults may be removed at common putting green mowing heights, and the effect is increased as mowing height is decreases. Very few adults were removed at collar or fairway height (> 7.62 mm). The majority of adults survived the act of mowing in all mowing height treatments, indicating the potential for adults to reinvade areas adjacent to where grass clippings are disposed. Laboratory and field experiments indicate that adult oviposition behavior is influenced by the height of the host plant. Significantly more eggs were placed outside of the turfgrass stem as mowing heights decreased. However, larval growth and development did not appear to be affected by mowing height, as significant numbers of larvae were capable of developing to damaging stages. My findings suggest that *L. maculicollis* pose a threat to putting greens. However, the threat of damage and need for insecticide applications may be lessened with increasing intensity of mowing practices (e.g. low mowing heights and increased frequency of mowing).
Introduction

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is considered the most devastating turfgrass insect pest of low-mown turf in the northeastern and Mid-Atlantic United States and parts of the southeastern provinces of Canada (Vittum et al. 1999; Simard et al. 2007). Damage is particularly severe in turfgrass stands with high percentages of *Poa annua* L. However, reports of damage to creeping bentgrass (*Agrostis stolonifera*) are becoming increasingly more common, especially as the insect expands its distribution into new regions where *P. annua* is less prevalent (Kostromytska and Koppenhöfer 2014; Kostromytska and Koppenhöfer 2016). These two turfgrasses represent a majority of the turfgrasses that may be found in the highest-valued turfgrass areas on a golf course. Therefore, insecticides are sequentially applied to these areas throughout the growing season to prevent larval damage. Superintendents make an average of four insecticide applications per year to keep populations from reaching damaging levels, though many report as many as six to 10 in a single season (McGraw and Koppenhöfer 2017). Overuse of broad-spectrum, preventive adulticides, particularly the pyrethroids, has resulted in a decrease in the susceptibility of some *L. maculicollis* populations to multiple classes of insecticides (Cowles et al. 2008). Rapid increase in prevalence of resistant populations has resulted in an urgent need for alternative controls.

*Listronotus maculicollis* adults overwinter in protected habitats (e.g. leaf litter, tall grass) surrounding the playing surfaces (Vittum et al. 1999). In spring, adults walk on to short-mown turfgrass areas to feed and mate (Diaz and Peck 2007). The female will find a suitable host, chew a notch in the stem of the plant, and insert the eggs into the stem or under the leaf sheath (Vittum 1980). The developing larva will bore through the plant as it develops through its first three instars. Once the fourth-instar is reached, the larva will exit the stem of the plant to feed externally on the crown. This will often sever the shoots from the roots, resulting in the death of the plant. The insect may have one
to three generations per year, though damage in spring from 1st generation late-instar larval feeding is usually most severe (Vittum et al. 1999; McGraw and Koppenhöfer 2007).

Damage is most severe on short-mown (< 1.25 cm) playing surfaces, including tees, fairways, and collars (the turf immediately bordering the surface). However, damage is rarely observed in putting greens, which are maintained at even lower mowing heights (2.25-3.8 mm) and provided with frequent fertilization, topdressing (inclusion of fine sand particles into the canopy and thatch layer) and fungicide applications. It is not uncommon to observe larval feeding damage on a green’s collar though not within the interior of the green. This may suggest that adults are impacted by the act of mowing prior to egg laying, or that larvae are incapable of developing in plants maintained below 3.8 mm. Mowing is essential to maintaining a smooth playing surface. Therefore, mowing is performed first thing in the morning 6 to 7 times per week on the golf course. The effect that mowing has on L. maculicollis growth and development remains largely unstudied though may prove effective as a non-chemical control option. Mowing has been shown to impact other turfgrass insects that lay eggs in putting surfaces. The black cutworm (Agrostis ipsilon Hufnagel), a major pest of bentgrass greens in the transition and cool-season regions of the US, lays its eggs on the distal end of the plant. As many as 91% of eggs have been shown to be removed when greens were mowed (< 3.2 mm) and 97% when combined with other management practices such as rolling and clipping removal (Williamson and Potter 1997). Golf course superintendents currently monitor grass clippings to assess L. maculicollis movement on playing surfaces (McGraw and Koppenhöfer 2017). Although mowing may be an effective monitoring tool, it remains unknown as to the ability of L. maculicollis to survive, oviposit, and develop in golf course putting green turf.

I investigated the potential for L. maculicollis to damage low-mown P. annua found on golf course putting greens as well as determine why damage is a rare occurrence in these areas. The
objectives of this study were to 1) determine the probability of *L. maculicollis* adults surviving putting green mowing 2) determine the ability for adults to oviposit in putting green-height turf 3) and to determine if larvae can develop to damaging stages in putting green mowing heights. It is my hope that the findings assist in reducing insecticide inputs on putting greens, and assist in the development of *L. maculicollis* best management practices (BMPs).

**Materials and Methods**

**Insects**

Adult *L. maculicollis* used in laboratory and field experiments were collected using a reverse leaf-blower vacuum (Echo ES 255, Lake Zurich, IL) from insecticide-free golf course fairways located in central Pennsylvania (Lancaster Country Club, Lancaster, PA; Sinking Valley Golf Club, Altoona, PA, Bucknell University Golf Course, Lewisburg, PA) and New York (Sleepy Hollow Country Club, Briarcliff Manor, NY) using the techniques described by McGraw and Koppenhöfer (2009). Insects used in Experiment 1 (see below) were collected from golf courses between 25 June and 1 July, 2015 and on 27 June, 2016. Insects used in Experiments 2 and 3 were collected during the initial overwintering-adult migration on to the short turf in spring in 2015 (10 April-14 April) and 2016 (18 April-22 April). Adults were placed in plastic containers fit with mesh lids in groups of 50-100. The containers were placed into a cooler and transported to the Turfgrass Entomology Laboratory at Pennsylvania State University (University Park, PA) where adult viability was assessed upon arrival. The insects were placed in a new container with a moistened paper towel. The containers were held in an incubator at 23°C (2015) or 10°C (2016), with a 12:12 light: dark cycle.
Experiment 1: Effects of putting green mowing height on *L. maculicollis* survival

A subset of the weevils (100) were measured using a Dino-Lite® Pro (AnMo Electronics Corporation, Hsinchu, Taiwan) digital microscope to determine clearance under a greens mower. Adults were placed into a freezer for 24 hr., and then pressed into Play-Doh® (Hasbro, Pawtucket, RI) to ensure accurate measurements. The average length of the adults from the back of the abdomen to the tip of the snout was 3.18 mm (± 0.02). Average width, from the top of the elytra to the bottom of the thickest part of the abdomen was 1.14 mm (± 0.01).

The ability of adult *L. maculicollis* to survive mowing at golf course green heights-of-cut was assessed in controlled greenhouse studies. Turf used in this experiment was removed from a *P. annua* research putting green (Land Management Resource Center, University Park, PA) immediately following mowing. Treatments consisted of one of three putting green mowing treatments (2.54, 3.18, 3.81 mm) representing a range putting green height-of-cut found across the region, and was mowed daily. In 2016, collar- (6.35 mm) and fairway-height (12.7 mm) treatments were added. These treatments were mowed three times per week. Cores (7.62 cm diameter × 4.8 cm depth) were removed using a turf plugger (Turf Tec International, Tallahassee, FL), and placed into plastic bags for transport to the laboratory. The turfgrass samples were transferred into plastic containers of the same dimension. The soil surface of the turf sample was made level with the upper rim of the container so that the height-of-cut could be accurately measured from the soil surface to top of the mower bed knife.

Adults were marked with ultraviolet (UV) ink (Dykem, Olathe, KS) so they could be tracked during mowing experiments. Adults were placed in the incubator for one hour after marking to allow the UV ink to dry. After the holding period, viability was assessed and weevils were placed in groups of five on a turf core. The cores were placed into incubators (23°C) for 24 hrs. to allow the weevils to acclimate to their environment and settle into the turf canopy. The 24-hr. period also served as a
realistic representation of the time between mowing events on a putting green. The following day, the turf samples containing *L. maculicollis* adults were taken to a greenhouse where mowing treatments were applied.

Experiments were conducted using a battery-powered, bench-mounted reel mower. The mower was suspended on a track over a greenhouse bench. Prior to each experimental run, the mower height was calibrated using a dial micrometer, measuring from the rim of the container (or soil surface) to the top of the mower’s bed knife. Adjustments to the height-of-cut were made by adding or removing sheets of computer paper under the turf sample. The order in which mowing treatments were applied was randomized after each treatment. Each mowing treatment was replicated 10 times per experiment, using a new core and weevils for each experimental run. Three experiments were conducted in both 2015 and 2016, for a total of 60 replicates per treatment.

Mowing treatments began by running the reel mower over a turfgrass core without weevils to ensure that the machine was properly functioning. The second pass was performed with turfgrass cores containing weevils. The mower was fit with a basket in front of the reel to capture clippings and any weevils that were ejected by the blades. Adult mortality was based on the number of weevils collected through a combination of manual examination of the turf core and clippings, followed by the use of a UV light to detect the marked weevils, and, where necessary, saline solution extraction. Adults that were collected alive, but were injured by the mower (damaged or cracked elytra) were recorded as dead. All experiments were conducted between 1100 and 1430 hr.

**Experiment 2: Effects of mowing height on *L. maculicollis* oviposition in no-choice assays**

A no-choice field experiment was conducted to determine if mowing height has an effect on *L. maculicollis* host plant acceptance. *Poa annua* cores were collected from research putting greens maintained at three heights (2.54, 3.18, 3.81 mm) using a soil core sampler (2.8 cm diameter × 9 cm
depth). In 2016, a fourth treatment (7.62 mm) was included to represent the mowing height found on putting green collars. The entire area was enclosed with a linear pitfall trap to ensure that *L. maculicollis* populations did not move into the plots and to prevent adult movement between plots.

Turf plugs were removed from individual mowing treatments and placed into a 50 mL conical tubes (Corning, Corning, NY). Sand was placed under the plug to ensure that the surface of the turf was even with the 40 mL mark on the tubes and ensure uniformity between samples. Adult weevils were placed into conical tubes (1 male and 1 female). A hole was drilled in the cap of the conical tube and fit with a fine mesh (324-mesh, 7.2 × 7.2 openings per cm²) to prevent adults from escaping. Tubes were driven into the ground so that the top of the turfgrass inside the conical tube was flush with the surface of the existing turf (Appendix ). After 48 hours, the adults were moved to a new conical tube containing a fresh-mown sample to ensure that females were provided with hosts of the same height-of-cut throughout the experiment. At this time, the old plugs were brought to the lab where the *P. annua* tillers were inspected for eggs using a light microscope (10x magnification). The number and the location of eggs (e.g. loose, within the stem) were recorded. A total of 20 mating pairs were observed for each mowing treatment. Each mating pair was observed for 18 days (9 egg inspections), or the length of time that most eggs are oviposited in captivity (McGraw unpublished data).

**Experiment 3: Effects of height-of-cut on *L. maculicollis* larval growth and development**

Larval development in golf course putting green turf was assessed in controlled field studies. Mowing height treatments of three greens height-of-cut (2.54, 3.18, 3.81 mm) and collar height-of-cut (7.62 mm) were separated from one another by linear pitfall traps and replicated 9 times. Field-collected adult *L. maculicollis* (1 male + 1 female) were contained in PVC arenas (12 cm height × 10 cm diameter) within a single mowing height replicate (60 cm x 180 cm). The enclosures were driven 2 cm into the ground within replicates. The containers were covered with a mesh (324-mesh, 7.2 × 7.2
openings per cm²) screen to prevent weevils from escaping. Each treatment replicate held 14 PVC enclosures.

Adults were allowed to oviposit in arenas for 50 growing degree days (simplified sine method with a base of 50°F) as calculated by an onsite weather station (WatchDog 2400, Spectrum Technologies, Aurora, IL). After this period, the containers and adult weevils were removed, and mowing treatments resumed. Two arenas from each replicate were removed weekly for seven weeks. Each core was inspected by either high heat extraction (early-instar larvae) or manually combined with saline extraction (older larvae). Early-instar larvae were too scarce to perform statistical analyses or obtain accurate assessment of weight. Therefore, they were omitted from the results. Larval abundance was obtained from 4th and 5th instars, and pupae recovered. Weights were obtained from 5th instar larvae only, which were pooled for each treatment, and weighed.

Statistical analyses

Statistical analyses were conducted using Statistix 9.0 software package (Tallahassee, FL, USA). One-way analysis of variance (ANOVA) was used to determine the effects of mowing height on L. maculicollis adult survival, oviposition, and larval development. Significance was determined at α = 0.05 level. Where significant differences were detected, means separation was performed using Tukey HSD all-pairwise comparisons. In Experiment 1, the average number of adults removed by the mower was compared by each of three (2015), or five (2016) mowing height treatments. The number of intact (alive), dead, and total adults were compared by mowing treatment. The effect of height-of-cut on the total eggs, as well as the location (loose, in-stem) was assessed for each of the three (2015) or four (2016) height-of-cut treatments in no-choice egg laying assays (Experiment 2). Finally, means separation was performed on the average weight and total number of L. maculicollis larvae found in each of the mowing treatments.
Results

Experiment 1: Effects of putting green mowing height on *Listronotus maculicollis* survival

Significantly more weevils were removed from the lowest mowing height (2.54 mm) than from the medium (3.18 mm) and high (3.81 mm) treatments (*df* = 2; *F* = 51.7; *P* < 0.01) in 2015 (Fig. 1.1). On average, the low treatment removed approximately 1.5 of the 5 adults per container (30%). Medium (7%) and high (2%) mowing treatments had minimal effect on removal. Additionally, significantly more weevils were collected alive from the lowest mowing treatments than compared to the other heights-of-cut (*df* = 2; *F* = 36.2; *P* < 0.01). No differences were detected between in the number of adults that were killed by the mower for low (2%), medium (2%) or high (4%) mowing treatments (*df* = 2; *F* = 1.68; *P* = 0.20).

The percentage of weevils removed increased with a decrease in mowing height. Significantly more weevils were removed from the lowest mowing height than from all other mowing heights tested in 2016 (*df* = 4; *F* = 3.33; *P* < 0.01) (Fig. 1.1). On average, the low treatment removed approximately 1.9 of the 5 adults per container (38%). Medium (13%) and high (7%) treatments had minimal effect on removal, although a greater percentage was collected from both treatments compared to 2015 experiments. All greens mowing heights (2.54-3.81 mm) collected significantly more adults than both the collar and fairway mowing heights (< 1% removed). There was no statistical difference between the number of adults killed by the mower in green treatments height-of-cut (*df* = 2, *F* = 1.69, *P* = 0.19). In general, adult mortality was low (ranging between 1-4%). No adults were killed at collar or fairway height, yet statistical differences were detected between these treatments and the lowest mowing height treatment (*df* = 4, *F* = 4.42, *p* = 0.02).
Experiment 2: Effects of mowing height on *L. maculicollis* oviposition in no-choice assays

Adult *Listronotus maculicollis* were capable of ovipositing in all three mowing treatments in no-choice field studies (Fig. 1.2). In 2015, significantly more eggs were laid in the low and medium green heights than in the highest height-of-cut ($df = 2; F = 0.78; P = 0.04$). The average number of eggs laid per treatment over the 48 hr. period was 1.78, 1.24, and 0.86 eggs, for the low, medium, and high height-of-cut, respectively. However, significantly more eggs were oviposited loosely (outside of the stem) at the lowest mowing treatment than compared to medium and high treatments ($df = 2; F = 4.65; P = 0.01$). There was no statistical difference between the number of eggs laid loosely at the medium and high greens height-of-cut. However, there was a trend toward increases in the mean number of eggs laid loosely as the height-of-cut decreased. No significant differences were detected in the number of eggs laid in the stem between treatments ($df = 2; F = 3.37; P = 0.46$).

Similar to experiments conducted in 2015, adults were capable of oviposition in all greens height-of-cut. No statistical differences were detected between the average number of eggs laid in putting green or the collar-height treatment that was added in the 2016 experiments ($df = 3; F = 0.48; P = 0.70$). The average number of eggs laid per treatment over the 48 hr. period was 1.34, 1.68, 2.41, and 2.35 eggs, for the low, medium, high, and collar height-of-cut, respectively. More eggs were oviposited on the exterior of the plant as mowing height decreased. However, there was no significant difference in the average number of eggs laid loosely between treatments ($df = 3; F = 1.49; P = 0.22$). Significantly more eggs were laid inside of the stem in the collar-height treatment when compared to the lowest putting green height ($df = 3; F = 3.70; P = 0.02$). There were no statistical differences between the average eggs laid inside of the stem between the three greens height-of-cut.
Experiment 3: Effects of height-of-cut on *L. maculicollis* growth and development

Mowing height did not appear to affect *L. maculicollis* larval abundance (Fig. 1.3). There were no statistical differences between the average number of 4th and 5th instars, pupae, or total stages recovered by mowing treatment ($df = 3; F > 0.12, P > 0.50$). Additionally, there were no significant effects of mowing height on larval fitness as measured by weight gain for fifth-instar larvae ($df = 3; F = 0.14; P = 0.94$). Fourth-instar larvae and pupae were not found in great enough abundance to be weighed because manual extraction was timed around greatest abundance of fifth-instar-larvae. On average, fifth instars weighed 6.4mg, 6.9mg, 6.6mg, and 6.6mg for low green, medium, high green height-of-cut, and collar height-of-cut, respectively.

Discussion

The results from laboratory and field studies demonstrate that *L. maculicollis* adults are capable of surviving low mowing heights, readily accept the shorter host plants, and larval development is unaffected by mowing height. This suggests that *L. maculicollis* pose a threat to golf course putting greens, despite damage being rarely observed in these areas. However, mowing may have a significant impact on *L. maculicollis* population dynamics in putting greens. Adults deviated from oviposition behavior that has been observed in previous studies conducted with higher-mown turf, as they were observed to place eggs loosely within the canopy at low mowing heights. The fate of these loose eggs remains unknown, but the vulnerability may make them more susceptible to predation. Additionally, moderate numbers of adults are removed with individual green mowing events. The cumulative effect of being removed by mowers may lead to significantly diminished population densities on putting greens.

Damage from late-instar larval feeding is not commonly observed within putting greens (McGraw and Koppenhöfer 2017). I hypothesized that this may be due in part to adults being removed
by the act of mowing, since damage is commonly observed in adjacent green’s collar that receive the same cultural inputs with the exception of mowing. Greenhouse studies indicated that moderate amounts (30–40%) of adults might be removed from greens that are mowed to a low height (2.54 mm). This effect may seem minimal in single mowing events, but considering that mowing occurs daily, or multiple times per day, the impact on adult abundance may be significant. However, the effect of mowing is likely to diminish with increasing mowing heights. Less than 1% of total infested adults were removed from collar and fairway height-of-cut. This may explain why damage is not observed on low-mown putting greens, yet is common on collars and fairways.

Mechanical removal with mowers may reduce *L. maculicollis* densities below damaging thresholds, particularly on low mown putting greens. Mowing has been successfully used as a mechanical control method for *A. ipsilon* on creeping bentgrass putting greens (Williamson and Potter 1997). Unlike *L. maculicollis*, black cutworm moths oviposit on the distal ends of the turfgrass leaf blades. Mowing putting greens (< 4.8 mm) was shown to remove as much as 91% of *A. ipsilon* eggs, and drastically reduced the potential for damage (Williamson and Potter 1997). Although the mowing treatments in this study did not remove the same percentage of adult *L. maculicollis* as, greens are mowed 6-7 days per week, and thus the cumulative effect on *L. maculicollis* populations may inhibit population establishment in these areas. Increasing the frequency of mowing in spring, when overwintering adult abundance is greatest may lead to even greater reductions in adult density. Double cutting is a practice in which greens are mowed twice in one event. The second cut serves to increase the smoothness and speed of the putting surface by removing leaf blades that were not mowed in the first pass (Karcher et al. 2001). The practice of double cutting requires increased labor and may be stressful to the turf. Therefore, double cutting cannot be practiced constantly throughout the year. However, future studies may wish to investigate the impact that double cutting has on adult *L.*
maculicollis abundance in golf course greens in the spring when cool-season turfgrasses are growing vigorously and under less stress than in other times of the year (e.g. summer).

Greenhouse studies demonstrated that the majority of adult L. maculicollis removed from putting greens by mowers are not killed. Much like L. maculicollis adults, A. ipsilon eggs are capable of surviving mowing practices. This suggests that in both cases, the need to remove clippings far from high-valued turf is critical to avoiding re-infestation. Generally, clippings are spread in the rough-mown turf surrounding the putting green, or combined with all turfgrass clippings in a central location. Adult survival in clippings suggests that disposal sites may harbor significant numbers of adults that may invade surrounding turf areas. Turfgrass clippings containing live L. maculicollis need to be discarded great distances from fine turfgrass stands, as L. maculicollis are capable of traveling hundreds of meters to find suitable host plants (Diaz and Peck 2007). Alternatively, future studies may seek to determine the effect that managing clipping piles (e.g. composting, burying) has on L. maculicollis survival.

Although L. maculicollis is capable of laying eggs in all low mowing height treatments, significantly more eggs were laid outside the turfgrass stem as putting green heights decreased. Most (83%) eggs found the collar height-of-cut were placed inside of the stem. Eggs laid outside of the plant on putting greens are likely to be much more vulnerable to predation and environmental extremes (e.g. heat, desiccation) than eggs that have been oviposited inside of the turfgrass stem. Numerous predators inhabit putting greens, including ground and rove beetles, and ants (Lopez and Potter 2000). The turfgrass ant (Lasius neoniger Emery) is a major predator of golf course insect pests, including A. ipsilon and Popillia japonica Newman eggs. Lopez and Potter (2000) found that L. neoniger was responsible for removing approximately 30 and 40% of A. ipsilon and P. japonica eggs from golf course fairways, respectively. The impact that L. neoniger had on A. ipsilon egg removal was even greater on putting surfaces as approximately 65% reductions were observed in a 22-hour period. It is unknown as
to the impact that predators have on *L. maculicollis* eggs in golf course putting greens. However, predation may contribute to the inability for populations to establish in putting surfaces, particularly on courses with low mowing heights. Golf course superintendents often consider *L. neoniger* to be a nuisance pest. Ant mounds can dull mower blades and smother turf mowed at a low height-of-cut (Potter 1998; Werle and Vittum 1999). Mounding can also compromise the smoothness of the playing surface (Maier and Potter 2005). Many superintendents will make insecticide applications targeting *L. neoniger* in an attempt to prevent mounding on high value turf areas (e.g. greens, tees, fairways) (Maier and Potter 2005). These early-season insecticide applications targeting *L. neoniger* and/or *L. maculicollis* on putting surfaces may diminish the impact of beneficial insect communities.

The results of laboratory and field studies provide insight into understanding *L. maculicollis* ovipositional behavior and the potential for damage to short-mown turf. Adults were shown to be capable of ovipositing in the lowest putting green mowing heights tested, and larvae were capable of developing to damaging stages. However, mowing was shown to remove a moderate amount of adults from the turfgrass canopy, both alive and dead. The ability of *L. maculicollis* to survive mowing suggests that golf course superintendents need to be aware of the placement of putting greens clippings. Combining these findings may lead to management practices that could reduce the amount of insecticides needed to control *L. maculicollis* populations on putting greens. Future studies need to address the effects that other putting green management practices (e.g. topdressing, irrigation, fungicide applications, plant growth regulation) and natural enemy complexes have on *L. maculicollis* behavior and population development.
Works Cited


Simard, L., J. Brodeur, and J. Dionne. 2007. Distribution, abundance, and seasonal ecology of *Listronotus maculicollis* (Coleoptera: Curculionidae) on golf courses in Quebec, Canada. J. Econ. Entomol. 100: 1344-1352.


Appendix. Conical tubes (50 mL) used to monitor the effects of adult *L. maculicollis* egg laying behavior in putting green and collar height-of-cut *P. annua*. 
Figure 1.1. Average *L. maculicollis* adults collected (alive and dead) by mowing treatment in greenhouse studies. Bars with the same letter within the same colored bars are not significantly different from one another at $\alpha = 0.05$ level (Tukey all pairwise comparison). Uppercase letters represent *L. maculicollis* collected alive and lowercase letters represent those that were killed by mowing.
Figure 1.2. Average *L. maculicollis* eggs laid loosely and inside the stem in no-choice oviposition studies. Bars with the same letter above the same colored bars are not significantly different from one another at $\alpha = 0.05$ level (Tukey all pairwise comparison). Letters over the bars indicate means separation of total eggs laid between mowing treatments. Uppercase letters represent eggs laid within the stem of the plant and lowercase letters represent loose eggs.
Figure 1.3. Mean total number of *L. maculicollis* larvae, pupae and total immature stages recovered from three putting green and a collar mowing height-of-cut in no choice field studies. No significant differences were detected at any life stage.
Chapter 3: Diel activity of *Listronotus maculicollis* adults in golf course putting green-height turf

Abstract

Most animals exhibit a distinct activity pattern with regards to time of day. Diel behaviors in animals are usually a trade-off between foraging, energetic demands, and avoidance of predators. The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is a highly destructive pest of golf course turf in the eastern United States and Canada. The small and cryptic weevil inhabits short-mown turfgrass areas, where it moves in and out of the turfgrass canopy to feed, mate, and disperse to oviposition sites. Little is known about the insect’s foraging behavior or vertical movement within the canopy. In previous studies, I determined that mowing has a significant effect on adult behavior and survival in golf course putting greens. Moderate numbers of weevils can be removed through mowing when the insect is above or within the turfgrass canopy. In this study, I examined *L. maculicollis* diel patterns of adult movement on the top of the canopy as a means to determine when the insect was most susceptible to removal by mowers. No distinct diel pattern to adult movement emerged in laboratory trials when adults were held at constant temperatures between 5 and 25 °C. However, significantly more adults were observed on top of the canopy between 15 and 25°C than at temperatures below 10°C. A distinct diurnal pattern was observed in a laboratory trial design to simulate the fluctuations between temperatures observed in the field. Activity was low during the night or dark (10°C) cycles, and immediately increased once light cycle conditions (18°C) were initiated, indicating that temperature may play a larger role in vertical activity in the canopy. Field observation studies of marked weevils provided support for a strong effect of temperature. Peak activity in the field occurred when temperatures were between 10° and 18°C. Adult activity on top of the canopy was significantly correlated with temperatures in April and May when temperatures ranged between three and 18°C. However, studies conducted in June suggest that temperatures greater than 18°C may cause weevils to
seek the lower portions of the turfgrass canopy. These findings suggest that adult canopy activity is a function of temperature, and that coordinating mowing efforts with temperatures in spring may greatly influence the impact that mowing has on reducing adult populations on putting greens.

Introduction

Most animals exhibit a diel pattern, or increase in their activity with regard to time of day in preparation for changing environmental conditions (e.g. darkness, temperature) (Greiner 2006, Fedderwitz et al. 2014). Changes in the environment can affect animal behavior, resulting in periods throughout the day when certain activities are performed most efficiently. Several hypotheses have been offered to explain differences in diel patterns among individual animal species including increased predation success, or predator avoidance (Harmsen et al., 2011), increased mating success (Shi et al. 2003; Zavodska et al. 2012), and increased foraging efficiency (Cerezke 1994; Duan et al. 1996; Fedderwitz et al. 2014). A better understanding of a pest’s diel behavior may lead to improved control tactics for the pest.

The annual bluegrass weevil, *Listronotus maculicollis* Kirby, is considered one of the most devastating turfgrass insect pests of low mown golf course turf in the northeastern and mid-Atlantic regions of the United States, and eastern Canada (Vittum et al. 1999; Simard et al. 2007). Adult *L. maculicollis* overwinter in leaf litter and other protective areas adjacent to golf course playing surfaces (e.g. fairways, tees, putting greens). In the spring, adults will migrate (primarily by walking) hundreds of meters from overwintering sites to find a suitable host (Diaz and Peck 2007). Once a suitable host plant has been located, the female chews a notch into the leaf sheath on the turfgrass stem, and places one to several eggs under the sheath (Vittum 1980). The first three instars are stem borers. However, fourth- and fifth-instar larvae cause the greatest damage to the turf stand while feeding externally on apical meristem. Most golf course superintendents rely on preventive insecticides to avoid larval feeding
damage and maintain acceptable playing conditions in high-valued turfgrass areas. An overuse of synthetic chemical insecticides, particularly the pyrethroids, has resulted in many populations to exhibit a decrease in the susceptibility of *L. maculicollis* to this and other classes of insecticides (Ramoutar et al. 2009). Rapid increase in prevalence of insecticide resistant populations has resulted in an urgent need to develop alternative controls.

Previous research (Chapter 2) suggests that significant numbers of adults may be mechanically removed from within and on top of the turfgrass canopy when putting greens are mowed, and that the effect increased with decreasing mowing heights. The impact that mowing has on adult populations in short-mown turf may be greatest when the most amount of adults are present on top of the turfgrass canopy. Black cutworm populations (*Agrotis ipsilon*) have been significantly reduced on *Agrostis stolonifera* putting greens through mowing (Williamson and Potter 1997). Black cutworms oviposit on the distal ends of individual turfgrass plants in a similar location to where *L. maculicollis* adults may be found when active on the turfgrass canopy. Several factors (e.g. photoperiod, air temperature) may influence when adults are most active on in this region. In previous laboratory studies, *L. maculicollis* adult movement was shown to be greatest at temperatures of 20°C when compared to other temperatures ranging from 0-30°C (Rothwell 2003). This may indicate that *L. maculicollis* has high and low temperature thresholds at which their activity decreases. Understanding the factors that influence when adults are most active on top of the turfgrass canopy is essential to using mowing as a mechanical control of the pest.

Observation of insects in their natural settings can be a difficult task (Rice et al. 2015). The majority of insect species are most active during the night, which can make observing them difficult without the aid of mark and recapture, or UV light tracking techniques (Danilevsky *et al.* 1970; Laudenslayer 1997). This may be true for *L. maculicollis* as the current belief is that they are most active
at night and are not readily observed on the turfgrass canopy during the day. Additionally, the small overall size of adult *L. maculicollis* and their ability to move vertically within the turfgrass canopy may also be contributing to the belief that the pest is nocturnal. However, *L. maculicollis* diel behavior remains unstudied and the true diel patterns of the insect are unknown. The lack of an understanding of *L. maculicollis* diel behavior and the difficulty in observing their movement requires the use of novel marking, and observation techniques of the pest.

I investigated the effects of temperature and time-of-day on adult *L. maculicollis* vertical movement within the turfgrass canopy. The objectives of this study were to determine if temperature or photoperiod influence adult vertical movement in the turfgrass canopy. To achieve this, different marking techniques in the field and laboratory were employed to observe vertical movement of adults in the turfgrass canopy. The findings of this study may assist in improving mechanical and chemical control of adults, thus reducing insecticide inputs on golf courses.

**Materials and Methods**

**Insects**

Adult *L. maculicollis* were collected using a reverse leaf-blower vacuum (Echo ES 255, Lake Zurich, IL) from insecticide-free golf course fairways located in central Pennsylvania (Sinking Valley Golf Club, Altoona; Bucknell University Golf Course, Lewisburg) and New York (Sleepy Hollow Country Club, Briarcliff Manor) using the techniques described by McGraw and Koppenhöfer (2009). Insects used in Experiments 1 and 2, as well as the April and May observations of Experiment 3 were collected from golf courses during the initial overwintering-adult migration on to playing surfaces in spring 2016 (13 April). Collections were timed by using growing degree-day data and plant phenology (*Forsythia* spp. bloom) (Diaz and Peck 2007). First generation adults were collected between 18-22 June and used to complete the June observations of Experiment 3. Field-collected adults were placed in plastic containers (11 cm
dia. × 14 cm deep) in groups of 50-100. The containers were fit with mesh lids to prevent weevils from escaping, and were placed into a cooler to be transported to the Turfgrass Entomology Laboratory at Pennsylvania State University (University Park, PA). Adult viability was assessed upon arrival, after which live weevils were placed into a new container with a moistened paper towel. The containers were placed in a controlled environmental chamber (10°C; 12:12 light: dark cycle) until use in experiments.

**Experiment 1: Diel activity of L. maculicollis at constant temperature**

Observational studies using time-lapse photography were conducted to determine if there are periods of increased adult activity on top of the canopy, and therefore identify a temperature when adults are likely to be removed by mowing. The elytra of the adults were marked with an ultraviolet pen (UV) (Dykem, Olathe, KS) in the laboratory. Weevils were held for 1 hr. to allow the marks to dry and to assess any adverse effects of the mark. *Poa annua* cores (5.7 cm diameter × 3.8 cm depth) were placed inside plastic containers (5.7 cm diameter × 4.8 cm depth) so that the walls of the container extended 1 cm above the turfgrass canopy. The walls of the plastic container were painted with Insect-Slip® (Fluon, BioQuip Inc., Rancho Dominguez, CA) to prevent weevils from escaping the observation arena. Five marked *L. maculicollis* adults were placed onto *P. annua* cores. Adult movement was monitored using a time-lapse camera (Brinno TLC 200 HDR). The camera was positioned directly over the sample and focused to capture the entire core. A UV light source was placed over the observational arena so that the marked weevils could be captured on the camera during dark periods. The weevils were allowed to acclimate to the environment in the incubator for 24 hr. prior to recording. The camera took a picture once every 15 minutes for 24 hours. The video was analyzed at the end of the observation period and the total number of adults observed on top of the canopy at each 15-minute period was noted. The number of adults was averaged by hour for statistical analyses. Observations were conducted at five
constant temperatures (5, 10, 15, 20, 25°C) using a 12:12, light: dark cycle. The order of the treatments was randomized after each run. Each treatment was replicated three times.

**Experiment 2: Diel activity of *L. maculicollis* with differing dark and light temperatures**

A second laboratory trial was conducted to examine the effects of time-of-day on vertical adult movement using different light and dark cycle temperatures. The same methodologies used to assess diel activity of *L. maculicollis* at constant temperatures were employed in this study. Differences included a lower dark cycle temperature (10°C) and a higher light cycle temperature (18°C). These temperatures were chosen to represent the average low and high temperatures in central Pennsylvania during the time when adult *L. maculicollis* are most prevalent on low mown golf course turf (late April-early May). Twelve 24-hour observations were conducted.

**Experiment 3: Diel activity of *L. maculicollis* under field conditions**

Field studies were conducted on *P. annua* research putting greens at the Landscape Management Resource Center (University Park, PA) in spring of 2016. Eight observational arenas (30.5 cm × 30.5cm) (Appendix (B)) were constructed within turfgrass plots that had been maintained at 3.81 mm. Weevils were marked with a Sharpie Neon (Sharpie, Freeport, IL) pen in the lab. The weevils were monitored for three hours after marking to ensure viability. Landscape edging (Master Mark Plastics, Albany, MN) was used as an enclosure to observe *L. maculicollis* behavior. The edging was driven into the ground (2.5 cm) on all four sides of the plot, and the walls (10 cm high) were painted with Insect-Slip® to prevent weevils from escaping. Ten marked weevils were placed into each of the eight arenas. Weevils were allowed to acclimate for 12 hr. prior to the recording of the data.

Adult activity on the turf surface was assessed each hour for a 24 hr. period using a DSLR camera (Nikon D90, Nikon Corp, Japan) and off-camera flash (Sunpak PZ42X, Sea & Sea Sunpak Co., Kawaguchi,
Japan). The flash was fit with an excitation filter (NIGHTSEA Ltd., Lexington, MA) to turn it into a blue light flash. Additionally, a yellow blocking filter (NIGHTSEA, Lexington, MA) was placed over the camera to exclude reflected blue light and transmit only the fluorescence, resulting in super-excitation of the marked weevil. A custom-made photo box (30.5 width × 30.5 depth × 45.7 cm height) (Appendix (A)) holding the camera and flash was placed over each observational arena to block out sunlight. The camera and flash were threaded through holes in the lid of the box to ensure consistency of height between photos. Photos were taken for each arena by moving the set-up over the observational arena. Observations were made over three 24-hour periods throughout the growing season (26 April, 16 May, and 28 June). Temperature was recorded at each observation period using an onsite weather station (Watch Dog Mini Station 2400; Spectrum Technologies, Aurora, IL).

**Statistical analysis**

Statistical analyses were conducted using Statistix 9.0 software package (Tallahassee, FL, USA). One-way analysis of variance (ANOVA) was used to determine differences in *L. maculicollis* activity at each time interval for all adult activity experiments. Where significant differences were detected (α = 0.05), means separation was performed using Tukey HSD all-pairwise comparisons. Pearson’s correlation was performed to assess a relationship between temperature and *L. maculicollis* activity in the outdoor experiments (Experiment 3). A second-order polynomial regression analysis was conducted on the combined data from all three months in outdoor experiments (Experiment 3) to describe the relationship between adult activity and temperature.
Results

Experiment 1: Diel activity of *L. maculicollis* at constant temperature

No significant patterns were detected in *L. maculicollis* behavior concerning time-of-day in any of the temperature treatments ($df= 23; F> 0.28; P> 0.83$) (Fig. 2.1). However, strong significant differences were detected between temperature treatments ($df= 4; F= 83.8; P< 0.01$) when average number of adults was pooled across all observation periods (Figure 2.2). No significant differences were detected in canopy activity between the three highest temperature treatments (15, 20, 25°C), though significantly fewer adults were observed on top of the turfgrass canopy at 5° and 10° C. Adult *L. maculicollis* activity was significantly lower at 5° C compared to 10° C.

Experiment 2: Diel activity of *L. maculicollis* with differing dark and light temperatures

Adults were observed to display diurnal vertical movement in laboratory assays when temperature was adjusted for light and dark cycles (Fig. 2.3). The average number of weevils active on top of the canopy ranged from $0.25 \pm 0.34$ during much of the nighttime hours to $2.5 \pm 0.65$ at 1300 hours. Unlike laboratory assays with constant temperatures (Experiment 1), strong statistical differences were detected in the number of *L. maculicollis* on top of the canopy over 24 hrs. ($df= 23; F= 2.10; P= 0.01$). Activity increased linearly from 0700 hr. to 1300 hr. Adult activity on top of the canopy remained high throughout the light period though decreased between 1300 to 1600 hr. despite constant temperatures. The average number of *L. maculicollis* adults active in the canopy between 1100 and 1800 hr. ($2.19 \pm 0.67$ adults) was six times greater than that of the average observed during the dark cycle ($0.25 \pm 0.34$ adults).
Experiment 3: Diel activity of *L. maculicollis* under field conditions

Adult *L. maculicollis* showed varying activity on top of the canopy during the three separate field observations (Figure 2.4). Significant differences were detected in the average adults adult canopy activity over 24 hr. periods (df= 23; F≥ 6.89; P≤ 0.01). More adults were observed on turf surface during daylight hours compared to dark periods for April (2.30 vs 0.86), May (2.95 vs. 0.88) and June (1.40 vs. 0.84) (df= 1; F≥ 10.68; P≤ 0.001). Daytime high temperatures during the June observation ranged between 17° and 29° C, whereas the daytime temperatures in April and May ranged between three and 19° C. As the temperatures in June increased above 19° C, weevil activity decreased sharply and remained depressed for the remainder of the day.

A significant positive correlation between temperature and adult canopy activity was detected in April (y=-0.0027x²+ 0.155x+0.268; R² = 0.46; P < 0.01) and May (y=0.018x² - 0.459x+0.268; R² = 0.08; P=0.04). Adult canopy activity increased linearly with temperature between sunrise and 1400 hr. However, adult activity declined despite temperatures increasing or remaining constant. A weak negative correlation between *L. maculicollis* canopy activity and temperature was detected in June (y= 0.001x²- 0.088x + 0.516; R² = 0.1345; p= 0.08). Temperatures ranged between 17 and 29° C during the observation period and were much higher than April and May. Highest activity occurred at 0700 hr., and decreased sharply as temperatures increased throughout the day.

In all three months, peak activity occurred when temperatures were between 14° and 18° C independent of the time of day (Fig. 2.5). The relationship between adult canopy activity (all months combined) was best described by second order polynomial regression model, indicating that peak activity in relation to air temperature occurs when temperatures are between 16° and 17° C (y= -0.08+ 0.26x– 0.008x²; R²= 0.084; P< 0.01).
Discussion

Laboratory and field studies indicate that *L. maculicollis* adults display increased vertical movement within the canopy with increasing temperature, and that activity is greatest within a narrow temperature range (16°–17°C). Activity was greatest during daylight in field trials between the hours of 1000 and 1400. However, laboratory observations under constant temperatures showed no variation in diel activity, which calls into question the diurnal nature of the insect. It may be that the effect of temperature has a greater impact on canopy activity than time-of-day. The relationship between temperature and activity was not linear in lab or field trials. Minimal adult activity was observed between 5° C (Experiment 1) and 10° C (Experiment 2). Five data points in the May observations (Experiment 3) did not correspond with the rest of the data collected over the three 24 hr. observations in this experiment (Figure 2.5). These points show a significant increase in activity when temperatures were between 5 and 15° C. This may be explained by the fact that the adults in this experiment were infested into the observational arenas when temperatures were 6° C. This potentially resulted in the adults being in a state of immobilization on top of the canopy through the 12 hr. acclimation period. When the first photographic observations were made, temperatures had decreased to 4° C. As temperatures increased throughout the observational period, the immobile adults were able to move down into the canopy and behave normally.

High temperatures may also negatively affect *L. maculicollis* movement. This relationship is not as clear, since no differences were detected between 15 and 25° C in the laboratory. However, field studies indicate that activity declines as temperatures increase above 18° C. These contrasting results may be the result of the unnatural conditions replicated in the laboratory studies. In the lab, activity may have been lower when temperatures dropped below 10° C because the insects physically could not function at these temperatures. A potential explanation for high activity above 20° C in the lab is that as
temperatures increased to a point where the insects could function normally, the weevils were constantly trying to escape observational arenas.

Adult *L. maculicollis* canopy activity was consistent over all periods in climate controlled laboratory experiments. However, as the constant temperature decreased below 10°C, canopy activity was significantly less than the other temperature treatments. The Argentine stem weevil (*Listronotus bonariensis*), a close relative to *L. maculicollis* also shows increased activity with increasing temperature (Barker and Pottinger 1986). Adult *L. bonariensis* feeding increased with temperature increases from 5 to 45°C in field studies. However, unlike *L. bonariensis*, *L. maculicollis* adult activity declined when temperatures exceeded 18°C. This difference may be explained by the fact that *L. bonariensis* is native to a much warmer climate than *L. maculicollis* and has evolved to tolerate much higher temperatures in order to thrive in its native range. Insects native to temperate regions such as *L. maculicollis* have the ability to survive winters at freezing temperatures (Bale et al 2002). This trait results in an inability to thermo-regulate their body temperatures during periods of high heat. Many temperate insects must attempt to cool their bodies by seeking refuge from the sun, and slowing activity (Bale et al. 2002). This may explain why *L. maculicollis* are less active on the turfgrass canopy when temperatures exceed 18°C.

Outdoor observations using marked adults revealed a narrow optimal temperature range for adult *L. maculicollis* vertical movement. Previous studies in controlled laboratory environments demonstrated greatest horizontal movement around similar temperatures as to what was observed in this study. Adults that had been held at 20°C moved significantly greater distances, when compared to adults held at any of the other six observed temperatures (0°C, 5°C, 10°C, 15°C, 25°C, 30°C) (Rothwell 2003). As temperatures moved below or above 20°C, activity decreased significantly, similar to models of vertical movement developed in Experiment 3. Adult *L. maculicollis* activity in Experiment 1 was highest at temperatures of 15, 20 and 25°C, with no significant differences in canopy activity at any of these
temperatures. The difference between this and previous laboratory studies may be explained because earlier studies involved removing adults from a controlled environmental chamber for observation at 7°C (regardless of temperature treatment). Field observations of adult *L. maculicollis* canopy activity in relation to temperature showed a similar response compared to Rothwell (2003) laboratory studies. However, *L. maculicollis* showed peak activity at temperatures between 16 and 17°C, with activity decreasing on either side of this range. This discrepancy may be explained because field observations in this study were completed with exact temperatures, and Rothwell (2003) made observations in 5°C increments.

In the field, *L. maculicollis* showed minimal activity during nighttime hours. However, this is not consistent with what has been observed with other closely related pests. Most insects, including many weevil species show increased activity during night hours (Danilevsky *et al.*, 1970; Cerezke 1994; Duan *et al.*, 1996; Fedderwitz *et al.* 2014). Adult *L. bonariensis*, unlike *L. maculicollis*, are only active during periods of “dim light” in the spring and summer (Barker and Pottinger 1986). However, several weevil species exhibit diurnal behaviors (Seybold *et al.* 2012). Adult *H. abietis* and *P. strobi* are generally most active during daylight hours (Solbreck and Gyldberg 1979, McIntosh *et al.* 1996). However, both weevil species appeared to respond more to temperature than time of day. Flight times of *H. abietis* increased as air temperatures increase above 18°C. Activity of *P. strobi* appeared to be less confined to a specific time early in the season when temperatures were lower. Into the summer months, when daytime temperatures were very hot, *P. strobi* activity was restricted to dawn and dusk. As daytime temperatures increased, weevils descend to the leaf litter until temperatures decrease again at dusk (McIntosh *et al.* 1996). This could explain the activity patterns observed with *L. maculicollis* canopy activity in field studies.
In field studies, *L. maculicollis* activity appeared to respond more to temperature than time of day. In April and May, nighttime temperatures were below the temperature (15° C) that laboratory experiments suggest activity in decreased. As air temperature increased above 15° C to a more optimal range, *L. maculicollis* activity increased linearly. In June, peak adult activity occurred when temperatures were between 17 and 18° C for a short time after sunrise. Therefore, it is not possible to state that adults display diurnal activity. To determine the true diurnal or nocturnal nature of the insect, future studies need to investigate *L. maculicollis* canopy activity in the laboratory and field during periods of dim light at temperatures between 14-18° C.

Multiple insecticide applications are often used to control overwintering adults migrating on to playing surfaces in spring (April- May), prior to egg laying to prevent larval feeding damage (Ramoutar et al. 2009). Previous research (see Chapter 2) has indicated mechanically controlling adults through mowing may significantly reduce adult populations on putting greens and diminish the need for insecticide treatments. The findings presented in this chapter suggest that adults will be most active on top of the canopy (and thus susceptible to removal through mowing) when temperatures range between 14-18° C. Most golf course managers mow putting greens in the early morning hours in an attempt to have surfaces prepared prior to opening the course to golfers. Early morning temperatures in April and May in the northeastern United States are often well below this range. Mowing at this time may result in a missed opportunity for removing adults. The variation in early spring temperatures means that those wishing to practice mechanical control should be flexible with regard to temperatures, rather than time of day. However, more studies are needed to validate the field model and assess the impact that mowing on reducing adult densities in putting greens.

Understanding *L. maculicollis* vertical movement within the turfgrass canopy may also lead to more efficient scouting, and chemical control tactics. Sampling for adults is a crucial part of determining
the need for control and the proper timing of insecticide applications. The efficiency of the most common adult sampling techniques (vacuum sampling, irritants or disclosing solutions) may also be affected by temperature (Diaz et al. 2008; McGraw and Koppenhöfer 2009). Scouting for adults when temperatures are between 14-18°C, may provide a better estimate of population density and better determine risk. Similarly, employing the vertical activity model to insecticide applications may lead to improved control. Contact insecticides used to control adults (e.g. pyrethroids, organophosphates, oils) may fail to achieve adequate suppression of the insect if it does not come in contact with the insecticide. Improved chemical management combined with mechanical removal from low mown surfaces may result in a reduction of insecticide applications.

The results of laboratory and field studies provide insight into understanding L. maculicollis diel behavior. These findings may be used to strengthen adult management strategies through mechanical and chemical control. Doing so will aid in the reduction of L. maculicollis populations below damage thresholds as well as reduce the onset of insecticide resistance. Future studies need to address L. maculicollis activity in different regions that experience varying temperatures and photoperiods, as well as how mowing time and frequency effects adult removal from putting greens.
Works cited

**Analytical Software. 2008.** Statistix 9.0 user’s manual: Tallahassee, FL.


Rothwell, N. L. 2003. Investigation into *Listronotus maculicollis* (Coleoptera: Curculionidae), a pest of highly maintained turfgrass. PhD Dissertation, University of Massachusetts, Amherst, MA, USA.


Simard, L., J. Brodeur, and J. Dionne. 2007. Distribution, abundance, and seasonal ecology of *Listronotus maculicollis* (Coleoptera: Curculionidae) on golf courses in Quebec, Canada. J. Econ. Entomol. 100: 1344-1352.


Appendix. Custom-made camera box (A) constructed to block out light for capturing photos of marked *L. maculicollis*, and observational arenas in the field (B) (Experiment 3).
Figure 2.1. Average adult *L. maculicollis* observed on top of the turfgrass canopy at five different constant temperatures and equal light: dark periods in controlled laboratory studies. Weevils were marked with ultraviolet ink, and detected with the use of an ultraviolet light and time-lapse camera placed over the observational arena. Hours shaded in grey represent periods of darkness.
Figure 2.2. Average adult *L. maculicollis* observed on top of the turfgrass canopy at five different constant temperatures and equal light: dark periods in controlled laboratory studies. Data was pooled across all 72 hours by temperature treatment. Weevils were marked with ultraviolet ink, and detected with the use of an ultraviolet light and time-lapse camera placed over the observational arena. Bars with the same letter are not statistically different (Tukey all pairwise comparison).
Figure 2.3. Average adult *L. maculicollis* observed on top of the turfgrass canopy with different light and dark cycle temperatures in controlled laboratory studies. Weevils were marked with ultraviolet ink, and detected with the use of an ultraviolet light and time-lapse camera placed over the observational arena.
Figure 2.4. Average number of *L. maculicollis* adults on top of the canopy, and temperature over three 24 hr. observations throughout the growing season in field studies (Experiment 3). The number of fluorescent marked individuals detected with still photography determined the number of weevils active on top of the canopy. Marked weevils were identified with specialized lens and flash filters. Hours shaded in grey represent periods of darkness.
Figure 2.5. Average number of *L. maculicollis* observed on top of the canopy as it corresponds to temperature over three 24 hr. field observations (April-June 2016). The number of fluorescent marked individuals detected with still photography determined the number of weevils active on top of the canopy. Marked weevils were identified with specialized lens and flash filters.