Phenology and Management of Annual Bluegrass Weevil on Virginia Golf Courses

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ACADEMIC ABSTRACT

Annual bluegrass weevil (*Listronotus maculicollis* Kirby) (Coleoptera: Curculionidae) (ABW) is a major pest of annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.) on golf courses in the northeastern United States. The asynchronous life cycle makes managing ABW difficult, putting emphasis on scouting to achieve accurate insecticide timing and acceptable control. Little is known about the biology and management of ABW in Virginia's more temperate climate. Reported cases of ABW resistance to pyrethroids (IRAC Group 3) continues to grow in the northeast, yet no pyrethroid-resistance cases have been reported in Virginia outside of the metropolitan Washington, D.C. For this thesis, I confirmed the widespread distribution of ABW across Virginia with a survey of golf course superintendents. Two golf courses in southwestern Virginia were monitored weekly during the 2019 and 2020 growing seasons to determine the seasonal biology of ABW within this region. These data suggest that overwintering ABW emerge much earlier than described in the northeast, with adult weevil activity beginning in late February or early March. I observed three complete ABW generations, with a potential fourth generation. Soil plugs from the same two golf courses were used to compare the salt floatation and Berlese-Tullgren funnel methods of larval extraction. The methods were highly correlated ($R^2 = 0.7856$), suggesting either method is appropriate for ABW larval extraction. Bioassays conducted on adult ABW from the same two golf courses showed that field rate concentrations of the pyrethroid bifenthrin showed variable mortality ranging from 20% to 80% suggesting the presence of resistance genes in the population. A 100-fold rate of bifenthrin resulted in 100% mortality of ABW, however. Because cross-resistance has been reported among northeastern ABW populations, the common insecticide active ingredients chlorpyrifos (IRAC Group 1B: Organophosphate), trichlorfon (IRAC Group 1B: Organophosphate), λ-cyhalothrin (IRAC Group 3: Pyrethroid), α-cypermethrin (IRAC Group 3: Pyrethroid), imidacloprid (IRAC Group 4A: Neonicotinoid), and spinosad (IRAC Group 5: Spinosyn), were tested on ABW adults. In another bioassay, two organophosphates, trichlorfon and chlorpyrifos, resulted in significantly higher ABW mortality rates than all other labeled insecticides ($P < 0.0001$). Two other larvicides, spinosad and α-cypermethrin, also exhibited adult control, an important factor to consider for ABW management and preventing pyrethroid-resistance. These results provide valuable insight into the seasonal biology and management of ABW in Virginia and direction for further investigation into these populations.

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GENERAL AUDIENCE ABSTRACT

Annual bluegrass weevil (*Listronotus maculicollis* Kirby) (ABW) is a tiny, but damaging insect pest of cool-season golf course turfgrasses in the northeastern United States. As pest populations have spread southward, ABW has become an emerging pest in Virginia. The objectives of this thesis were to 1) determine the geographic distribution and seasonal biology of ABW in Virginia, 2) compare two methods for extracting ABW larvae from turf cores in order to estimate larval densities, and 3) to assess the susceptibility of ABW to various insecticides commonly used by golf course superintendents. A survey of golf course superintendents in 2019 revealed widespread prevalence of ABW across Virginia, particularly in areas growing predominantly cool-season turfgrasses. Two golf courses in southwestern Virginia were monitored weekly during the growing seasons of 2019 and 2020 to determine the seasonal biology of ABW. Data suggest that overwintering ABW emerge much earlier than reported in the northeast, with adult weevil activity beginning in late February or early March compared to April in the northeast. In addition, I detected three complete ABW generations, with a possible fourth generation occurring. Soil plugs from two golf courses were used to compare a heat extraction method using a Berlese-Tullgren funnel with the traditional salt float method for extraction of ABW larvae. The two methods extracted similar numbers of ABW larvae suggesting that either method is appropriate for ABW larval extraction. Adult ABW from the same two golf courses were collected and subjected to the pyrethroid insecticide bifenthrin at 0.128 kg ai ha-1 (field application rate), 1.28 kg ai ha-1 (10-fold rate), 12.8 kg ai ha-1 (100-fold rate), along with a water control. Results indicated that the field application rate of bifenthrin killed > 50% but <90% of ABW adults, suggesting that some resistance may be present in the population. A concentration of 100-fold bifenthrin field rate killed 100% of tested individuals, suggesting that widescale field resistance to pyrethroids is likely not present. Because cross-resistance has been reported among northeastern ABW populations, the common insecticide active ingredients chlorpyrifos (IRAC Group 1B: Organophosphate), trichlorfon (IRAC Group 1B: Organophosphate), λ-cyhalothrin (IRAC Group 3: Pyrethroid), α-cypermethrin (IRAC Group 3: Pyrethroid), imidacloprid (IRAC Group 4A: Neonicotinoid), and spinosad (IRAC Group 5: Spinosyn), were tested on ABW adults from the same two golf courses. Two organophosphates, trichlorfon, a larvicide, and chlorpyrifos resulted in significantly higher ABW mortality rates than all other active ingredients ($P < 0.0001$). Two other larvicides, spinosad and α-cypermethrin, also exhibited adult control, an important factor to consider when attempting to manage ABW and preventing resistance to pyrethroids. Our results provide valuable insight into the seasonal biology and management of ABW in Virginia and direction for further investigation into these populations.

Dedication

I would like to dedicate this thesis to my bright and bubbly baby sister, Riley Gallaher. Although you may not realize or understand right now the tremendous impact you have had on my life in your short seven years, I hope I can inspire you the way you have done to me. May your love of learning continue to flourish, and your moxie and effervescent personality continue to grow as you do. I hope that I can provide for you a role model and an unwavering backbone of support.

I would also like to dedicate this thesis to amazing parents, Brittney Gallaher, Paul Daly, and Dennis Gallaher, whom have had an undying and unwavering belief in my abilities and have supported me the entire way; as well as my loving Grandma, Mary Ann Daly, and Grammie, Maureen Clemons, who have never failed to show their love and belief in me. Without everyone's love and guidance, I would not be half the person I am today. I cannot express enough gratitude for everything they have done for me and taught me. I hope I have made all of you proud.

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List of Abbreviations

1. Annual Bluegrass Weevil (ABW)
2. Insecticide Resistance Action Committee (IRAC)
3. Median Lethal Concentration (LC_{50})
4. Golf Course Superintendents Association of America (GCSAA)
5. Blacksburg Country Club (BCC)
6. Ballyhack Golf Club (BHGC)
7. Growing-Degree Day (GDD)
8. Analysis of Variance (ANOVA)
9. Water Control Treatment (WCT)
10. High Density Polyethylene (HDPE)

Chapter 1. Literature Review

Introduction

Annual bluegrass weevil (*Listronotus maculicollis* Kirby) (Coleoptera: Curculionidae) (ABW) is an established pest of cool-season turfgrass on golf courses. It is the most damaging pest of cool-season golf course turfgrass in the northeastern United States (McGraw and Koppenhöfer 2008). Based on a survey published in 2017 (McGraw and Koppenhöfer 2017), on average, golf courses spent $9,270 on ABW management alone. While this weevil prefers to oviposit, develop, and feed on annual bluegrass (*Poa annua* L.), it can also develop on creeping bentgrass (*Agrostis stolonifera* L.) (Koppenhöfer et al. 2018). Annual bluegrass weevil is particularly damaging to short-mown areas of golf courses including tees, fairways, collars, and greens (McGraw and Koppenhöfer 2008). The use of traditional insecticides, such as pyrethroids and organophosphates, have been found to be the most effective means of control, as there are no significant cultural controls that can provide the level of control that chemical pesticides provide (Vittum 1999) and biological controls, such as entomopathogenic nematodes and *Bacillus thuringiensis*, have proved less effective than golf course superintendents are willing to tolerate (McGraw and Koppenhöfer 2017). Multiple insecticides, most recently chlorpyrifos and insecticides containing chlorpyrifos, have been banned in states such as Hawaii, California, Maryland, New York, Oregon, Connecticut, and New Jersey (Abbotts and Morales 2020). Although insecticides are the most effective way to control ABW, current and possible future legislation combined with known pyrethroid resistance (Ramoutar et al. 2009b, McGraw and Koppenhöfer 2017, Kostromytska et al. 2018) might ultimately limit the availability of effective insecticides that can control annual bluegrass weevil.

While the majority of ABW research has been conducted in the northeastern United States, little is known about ABW biology and the effectiveness of insecticides for its control in Virginia. Understanding how and when ABW develops and their response to insecticides in Virginia is vital for golf course management as this pest has become one of the most expensive insect pest problems the industry has ever seen (McGraw and Koppenhöfer 2017).

Overview of *Listronotus maculicollis*

Annual bluegrass weevil was first reported in Connecticut in 1931 (Britton 1931) and, until the 1990s, was concentrated around the metropolitan area of New York, including New Jersey and Connecticut (Cameron et al. 1968, McGraw and Koppenhöfer 2017). The pest has consistently expanded its distribution and range and has now spread to Quebec and Ontario in the north, western North Carolina in the south, and as far west as Ohio, and more recently Kentucky (Anonymous 2021), in the United States (McGraw and Koppenhöfer 2017). Annual bluegrass weevil has one to four generations per year depending upon geographic location and climate, with the most southern weevils exhibiting four generations and the most northern weevils exhibiting one (McDonald and Dernoeden 2007).

The adult ABW are black to grey in color and are approximately 3 mm long and have a distinct broad snout, like many Curculionids (McDonald and Dernoeden 2007, McGraw and Koppenhöfer 2008) (Fig. 1.1). When adults emerge directly after pupation, they are a reddish-brown color (Fig. 1.2), as their exoskeleton has yet to fully develop and will continue to darken with age (Beard 2002). During oviposition, adult female ABW will chew holes in the outer leaf sheath of the grass plant in order to insert their eggs, although this chewing damage is considered negligible (Vittum 1999). The eggs laid are oval in shape and about 0.25 mm wide and 0.8 mm long (Peck et al. 2007). Larvae pass through five instars on their way to pupation and range in

size from 1 mm to 4.5 mm depending on their developmental stage (Peck et al. 2007). Larvae are creamy white in color with brown enclosed head capsules and are legless (Fig. 1.3), making them distinguishable from white grubs, common turfgrass pests that are also creamy white in color but have six legs.

In the Northeast US, adults emerge from overwintering during warm periods from late March to early May. In the Mid-Atlantic region, adult ABW become active in late February to mid-March depending on the location and local environmental conditions (Billeisen 2017). Historically, emergence of overwintering adults has been correlated to plant phenological indicators including forsythia (*Forsythia* sp.) at 50% petal loss, dogwood (*Cornus* sp.) full bloom, and eastern redbud (*Cercis canadensis*) full bloom (Tashiro 1987, Diaz and Peck 2007, McGraw et al. 2020). However, plant phenological indicators often are not consistent with industry recommendations of application timing based on growing-degree-day (GDD) accumulation (Vittum and Brocklesby 2013) and can be variable between cultivars and even the same cultivars on the same golf course (McGraw et al. 2020). Shortly after emerging from overwintering, the female ABW lays two to six eggs inside the turfgrass plant sheath (McGraw and Koppenhöfer 2008). In a study conducted by McGraw et al., three weeks after adult emergence, all females collected for the study possessed well-developed reproductive systems (McGraw et al. 2020). The eggs then hatch into larvae and feed on the grass plant while moving down towards the crown of the plant. Once the crown of the plant is consumed, the larvae burrow down into the soil-thatch interface to continue to feed on the turfgrass roots until they are developmentally ready to pupate (Beard 2002). Once overwintered adults have emerged from their overwintering locations in tree litter, moss, and/or high-cut grass (Diaz and Peck 2007), they can quickly cycle through their generations and, often, multiple life stages are present

simultaneously (McGraw and Koppenhöfer 2008). As a result, effective application timing for management of ABW is challenging (Billeisen 2017).

Annual bluegrass weevil larval feeding damage (Fig. 1.4) on closely mown golf course turfgrass which first appears as small yellow-brown spots (Vittum 1999). Due to the consumption of the crown by the larvae, the entire grass plant the larvae have been feeding on most often turns completely tan-yellow or dies, leaving behind the small yellow-brown spots described by Vittum (1999). When crown feeding occurs, the turfgrass plant can easily be pulled from the soil and will often exhibit a hollowed grass stem, a key diagnostic feature of ABW (McDonald and Dernoeden 2007). Damage caused by ABW is most apparent during the summer months and is often confused with drought or heat stress of *P. annua* and *A. stolonifera* along with the natural dieback process of *P. annua* (McDonald and Dernoeden 2007).

Different insecticide chemistries are used to treat the ABW adult and larval stages; most often, pyrethroids, IRAC Group 3 are used to control ABW adults (Cowles et al. 2008), while diamides, organophosphates, or spinosyns typically control the larval stage (McGraw and Koppenhöfer 2017).

Annual Bluegrass Weevil Pest Management

Chemical Control

Insecticides are required to control ABW at a level that meets the standards often expected from golf course superintendents (Vittum 1999). Due to the asynchronous life cycle and multiple yearly generations of ABW, golf course superintendents often use multiple chemistries to treat both the ABW adults and larvae throughout the activity season (Table 1.1). The insecticide classes that are used to control adult ABW are organophosphates and pyrethroids (McGraw and Koppenhöfer 2017). The most common active ingredients within these classes are

bifenthrin, λ-cyhalothrin, deltamethrin, and chlorpyrifos. Anthranilic diamides, carbamates, neonicotinoids, organophosphates, oxadiazine, and spinosyn insecticide classes are used to treat the larval ABW stages (McGraw and Koppenhöfer 2017). The most common active ingredients with larvicidal activity include trichlorfon, imidacloprid, spinosad, indoxacarb, and chlorantraniliprole. Combination products are also available that are designed to treat and control both the ABW larvae and adults at the same time with one product that implements different classes of insecticides or active ingredients. In a survey conducted by McGraw and Koppenhöfer (2017), it was found that pyrethroids and chlorpyrifos, an organophosphate, were the two most popular means of controlling ABW adults. Although the number of cases of ABW resistance to pyrethroids is growing, some golf course superintendents apply pyrethroids as their sole means of ABW adult control as they have expressed that pyrethroid insecticides cost much less than the other chemistries available on the market (McGraw and Koppenhöfer 2017). In the same survey, it was found that larvicides, especially chlorantraniliprole, indoxacarb (Provaunt, Syngenta), spinosad (Conserve, Dow AgroSciences), and trichlorfon (Dylox, Bayer) were used more often on courses reporting resistant ABW than on courses with susceptible populations, though many of these respondents (64%) still primarily used pyrethroids for ABW adult control (McGraw and Koppenhöfer 2017).

Annual Bluegrass Weevil Resistance to Pyrethroids

Past studies have demonstrated dramatic differences in ABW's susceptibility to pyrethroid insecticides in populations found in the northeastern US (Cowles et al. 2008, Ramoutar et al. 2009b, Ramoutar et al. 2009a, Koppenhöfer et al. 2018, Kostromytska et al. 2018, Koppenhöfer et al. 2019). Ramoutar et al. (2009a) determined the mechanism of pyrethroid resistance of ABW to be enzyme-mediated insecticide metabolic detoxification. In a

similar study (Ramoutar et al. 2009b), specific pyrethroid LD_{50} values for northeastern weevils were determined. The LD_{50} ranges for bifenthrin and λ-cyhalothrin, two pyrethroids, were 1.80-244.67 ng insect^{-1} and 0.52-159.52 ng insect^{-1}, respectively (Ramoutar et al. 2009b). When ABW strains collected from southern New England golf courses were tested, they showed low to high levels of resistance to bifenthrin (6.1-135.9 fold) and λ-cyhalothrin (28.7-306.8 fold) (Ramoutar et al. 2009b). Selection by pyrethroids has now left many courses with adult weevils that are unaffected by pyrethroids (Cowles et al. 2008). In a survey conducted by McGraw and Koppenhöfer (2017), they found that 20.1% of golf courses with damaging ABW populations (most distributed through New York, Connecticut, and New Jersey) reported having a pyrethroid-resistant population either suspected or confirmed by a bioassay.

Once pyrethroid resistance has developed in an ABW population, continued use of pyrethroids will only exacerbate the resistance (Cowles 2010). In field studies (Cowles 2010), slight increases in mortality when combining pyrethroids with synergists (such as piperonyl butoxide, demethylation inhibiting fungicides, and gibberellin-blocking growth regulators (Ramoutar et al. 2010b)) have not been statistically significant. Substituting pyrethroid use with selective larval insecticides can encourage predatory insects to keep ABW populations in check, instead of consequentially treating for them with pyrethroid applications (Cowles 2010).

Cultural Control

Although insecticides are fundamental to controlling ABW on golf courses, implementing cultural controls implemented alongside chemical insecticides can help reduce the number of applications needed during the activity period. Cultural control options include proper plant nutrition and irrigation, which can often help mask the symptoms of ABW damage (McDonald and Dernoeden 2007). Annual bluegrass weevil adults overwinter in tree lines,

under leaf litter, and in higher-mown turfgrass areas such as roughs (Diaz and Peck 2007, McGraw and Koppenhöfer 2007). Superintendents report that removing leaf litter regularly for three or more years seems to decrease the overall weevil population in the surrounding areas in New England (Vittum 1999). Because ABW favors *P. annua* over *A. stolonifera* for oviposition and feeding, any program that suppresses the *P. annua* within the turfgrass stand would possibly reduce the damage of the ABW (Vittum 1999). In contrast, enhancing the health of the *P. annua*, along with *A. stolonifera*, could possibly mask the symptoms and allow the *P. annua* to withstand damage (Vittum 1999).

Biological Control

Few marketable biological control products exist for use on ABW. The use of microbial control agents in turfgrass overall is limited due to high quality standards and competition from synthetic insecticides (Koppenhöfer and Wu 2017). The entomopathogenic fungi *Metarhizium* spp. and *Beauveria bassiana* are available for the control of surface feeding insects (Meyling et al. 2011), but there are few efficacy data available. Meyling et al. (2011) found most herbivores affected by the entomopathogenic fungi were coleopteran, however the percentage of all coleopterans effected was not determined. Another biological control option is entomopathogenic nematodes, microscopic roundworms found in the soils of most ecosystems (Cowles et al. 2008). In 2009, a study was conducted to evaluate the efficacy of two endemic and five commercial entomopathogenic nematode species as a control means of ABW (McGraw et al. 2009). The authors found that a mixture of two nematode species, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, achieved the highest levels of adult control at 50% mortality. However, these nematodes required 6 days to reach this mortality level and they were applied at five times the field application rate. Although nematodes only caused 50% mortality in adults,

there was much more control in the fourth and fifth instar larvae. *Steinernema feltiae*, *S. carpocapsae*, and *S. kraussei* significantly reduced fourth instars in both years of the study, however there was a large amount of variability in the reduction levels (37%-92%) (McGraw et al. 2009). The variability of results and requirements for adequate control pressure vastly limit the potential of biological controls for the current ABW insecticide market, further encouraging synthetic chemical insecticides for control of ABW.

Adult Sampling Methods

Irritant Sampling

The methods for effective ABW extraction have primarily been studied using other common insect pests. One way of sampling that has since been prescribed for ABW is that of irritant sampling, which was originally used to target soil-dwelling Lepidopteran larvae (Tashiro 1987). Irritant sampling involves a mixture of water and an irritant that is poured or sprayed over an area of turfgrass suspected of harboring pests. Traditionally, irritants included dry detergent, liquid detergent, or pyrethrin (Tashiro 1987, Hellman 1994). In a study conducted in 1983 (Tashiro et al. 1983), several concentrations of both liquid detergent (0.063%-1.000%) and pyrethrin (0.0004%-0.0060%) were tested at rates of 4 liters of mixture per 1,860 cm^2 of turfgrass. The authors found that 0.25% liquid detergent concentration and 0.0015% pyrethrin concentration were the most efficient and were implemented as the standard in following studies. It was also found that the liquid detergent mixture forced the Lepidopteran larvae to surface more quickly than the pyrethrin mixture. In the same study, the authors determined that continuous observation was essential for accurate larval counts.

While the use of pyrethrins for irritant flushing has been proven as a reliable method of sampling, many have moved away from their use in favor of detergent due to several factors. The

first factor is the death of young, non-target pests that also inhabit the soil (Hellman 1994). The variability in achieving correct concentrations and subsequent misapplication account for the second and third factors (Hellman 1994). Thus, irritant sampling utilizing liquid detergent is more common and more widely used today. Using this method requires thoroughly soaking the turfgrass soil surface and thatch layer. Soaking the desired turfgrass area forces the thatch- and soil-dwelling arthropods and annelids up to the surface to escape the irritant mixture. Escaping the thatch layer generally takes five to ten minutes to observe (Tashiro 1987, Hellman 1994). The standard soap concentration of the irritant mixture and the necessary collection time for ABW varies throughout literature, however. In one instance, a detergent concentration of 1-2% with a collection time of 10-15 minutes is recommended (McGraw and Koppenhöfer 2008). In a more recent study (Koppenhöfer et al. 2020), irritant flushing using liquid detergent was most effective with 5.2 L m^{-2} water containing 0.8% liquid detergent applied twice with adults being collected for 20 minutes.

Vacuum Sampling

The use of vacuum suction for insect sampling has been used most often in agricultural settings as a means of foliage sampling (Tashiro 1987, Yi et al. 2012). Within agricultural systems, vacuum sampling has been compared to other methods of foliage sampling such as sweep netting and whole plant collection and observation (Buffington and Redak 1998, Doxon et al. 2011). Doxon et al. (2011) found that when compared to sweep netting, vacuum sampling is more suitable for invertebrates smaller than five centimeters. There are two common types of vacuum sampling apparatuses, the Johnson-Taylor suction trap and the Dietrick vacuum insect net (D-vac) (Yi et al. 2012). Johnson-Taylor (Johnson 1950, Taylor 1951) suction traps are used primarily for catching aerial arthropods, and thus are not considered or utilized for ABW

sampling. The D-vac (Dietrick et al. 1960) is better suited for ground arthropods and usually consists of a motorized exhaust fan and a collection net. The D-vac is an efficient method which permits the sampling of relatively large areas within a short period of time (Yi et al. 2012). However, the traditional D-vac is considered bulky and can be expensive per unit (Yi et al. 2012, Zou et al. 2016). Because of this, some have turned to the modification of traditional leaf-blowers into vacuum samplers (McGraw and Koppenhöfer 2008, Zou et al. 2016) to sample ground arthropods. In a study of three adult ABW collection methods, irritant sampling with liquid detergent was most effective (83% recovery in fairways), while vacuum sampling and leaf clipping collection recovered only 31% and 24%, respectively (Koppenhöfer et al. 2020).

Pitfall Traps

Pitfall traps are a common means of sampling for ground-dwelling turfgrass pests including billbugs, mole crickets, chinch bugs, and other highly mobile arthropods (Lawrence 1982, Hellman 1994). They are also utilized for ABW sampling along golf course fairways (Diaz and Peck 2007, McGraw and Koppenhöfer 2008), although this is not as common. The traditional pitfall trap design is a small hole or pit lined with a container that contains a killing liquid, such as soapy water or alcohol, however this is not required (Hellman 1994, Hohbein and Conway 2018). The initial pitfall trap set-up is time consuming but allows for quick, season-long inspection. Often, a pitfall trap is best utilized for monitoring first occurrence or the initial emergence of adult weevils during the spring, as ABW adults move onto fairways by walking rather than flying (Hellman 1994, Diaz and Peck 2007). The most common variation of the traditional pit fall trap utilizes a plastic cup situated flush to the soil surface, making for a smaller and less obvious disturbed area (Tashiro 1987, Hellman 1994). Another pitfall trap variation includes a PVC pipe with a longitudinal slit cut into the surface-facing side and caps on each end

to prevent insect escape (Lawrence 1982, Tashiro 1987, Diaz and Peck 2007). Although pitfall traps are common in studies assessing arthropod abundance, 55.6% of studies utilizing pitfall traps did not specify at least one of the following pieces of information: container material, container diameter, preservative specification, or sampling interval (Hohbein and Conway 2018). This lack of specificity allows for many variations of the traditional pitfall trap, although these variations generally produce comparable results to the more traditional style (Yi et al. 2012, Hohbein and Conway 2018).

Although pitfall traps are reliable for initial overwintering emergence estimations, their use as a practical approach to estimate weevil abundance throughout the season is not common outside of research purposes (McGraw and Koppenhöfer 2008) and accurate damage predictions based on pitfall traps are not reliable (Hellman 1994).

Larval Sampling Methods

Salt Floatation Method

There are a variety of methods that can be employed when sampling for grass- or soil-dwelling arthropods, including ABW adults and larvae. One method that has been adapted over the years is that of floatation. Early floatation methods included the use of benzene or magnesium sulfate used in combination with air and water jets to force the desired arthropods to the surface (Salt and Hollick 1944). Newer adaptations of extraction via floatation substitute benzene and magnesium sulfate for common table salt (NaCl) (Sotherton 1984) which alters the density of the water, enabling the arthropods to float to the surface for counting and/or collection, with no additional use of air or water jets required. Not only does the salt change the density of the water, but it also irritates the ABW larvae causing them to float to the top in an effort to escape the salt water (McDonald and Dernoeden 2007). Multiple floatations of the same

sample are often needed to further separate organic matter from desired arthropods or materials (Sotherton 1984). For ABW specifically, larval sampling and extraction is often conducted via salt floatation in a lukewarm saturated salt solution (Koppenhöfer et al. 2018). This method recommends removing soil cores from the desired turfgrass area, breaking apart the soil cores, then submerging them in salt water for one hour (Koppenhöfer et al. 2020). While this method is common and takes relatively little active time, breaking apart the soil cores thoroughly without salt floatation to extract ABW larvae is also practiced (Vittum and Tashiro 1987, Ramoutar et al. 2010a), though more time consuming and tedious.

Berlese-Tullgren Funnel Method

An alternative method to salt floatation for the extraction of ABW larvae is that of heat extraction via a Berlese-Tullgren funnel. Berlese funnels were originally utilized by Antonio Berlese in soil extractions using a heated water jacket, maintained between 60ºC to 100ºC, as the driving source of heat (Berlese 1905, Owens and Carlton 2015). With his device, Berlese was able to easily extract small insects of all orders including myriapods, symphiles, pauropods, spiders, mites, and springtails (Berlese 1905). This heated water jacket created a flow of warm water that established a temperature gradient of 30ºC-35ºC throughout the soil core sample (Blasdale 1974). This Berlese funnel was later reimagined by Albert Tullgren, who replaced the water jacket with an incandescent lightbulb suspended above the sample (Tullgren 1918, Owens and Carlton 2015). Tullgren's changes improved the retention and distribution of heat, established a vertical heat and moisture gradient, but subsequently sped up the rate of desiccation (Tullgren 1918, Owens and Carlton 2015). Today, the latter configuration is commonly used to extract micro-arthropods inhabiting the soil (Yi et al. 2012), however, in some instances (Blasdale 1974), the use of a water driven temperature gradient is preferred.

Using the Berlese-Tullgren funnel method, soil cores are taken from the area(s) of interest and placed into the funnel where an incandescent bulb hanging above causes the temperature gradient (Tashiro 1987). This temperature gradient causes the arthropods within the soil cores to move from the heated end of the soil sample to the cooler end and down into a collection apparatus (Yi et al. 2012). This method has been used successfully for billbug adults, chinch bugs, webworms, cutworms, and mites (Niemczyk 1981). Although the use of a Berlese-Tullgren funnel for ABW larvae extraction specifically has not been studied, anecdotal evidence suggests it could be a viable means of extraction and sampling of ABW larvae (McGraw 2020).

Research Objectives

While the biology of ABW has been well studied in the northeastern U.S., little is known about ABW emergence and overwintering dates in the Mid-Atlantic US including Virginia. Similarly, the efficacy of pyrethroids for ABW control in Virginia is not known outside the metropolitan Washington, D.C. area. Furthermore, because the timing of insecticide applications for this pest is critical, accurate scouting or sampling for ABW larvae and adults is crucial. The objectives for this work were:

1. To determine the current distribution of ABW on golf courses in Virginia.
2. To determine the phenology of ABW populations from two Virginia locations.
3. To compare two techniques for extracting ABW larvae from soil core samples.
4. To evaluate the current susceptibility of ABW adults collected from Virginia golf courses to bifenthrin, a pyrethroid, and other commonly used insecticides.

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Tables and Figures

Figure 1.1. Adult annual bluegrass weevil relative to the size of an index fingernail.

Figure 1.2. Newly emerged (teneral) annual bluegrass weevil exhibiting a brown exoskeleton not yet fully developed. (Photo credit: GCM Online)

Figure 1.3. Annual bluegrass weevil larvae (circled) and pupa (boxed) collected from Blacksburg, Virginia.

Figure 1.4. Annual bluegrass weevil damage on a golf course putting green and collar. (Photo Credit: Jim Wilson)

Table 1.1. Insecticides labeled for annual bluegrass weevil control on turfgrass in the United States.

Active Ingredient	IRAC Group	Life Stage Treated
Carbaryl	1A: Carbamate	Larva
Chlorpyrifos	1B: Organophosphate	Adult
Trichlorfon	1B: Organophosphate	Larva
Bifenthrin	3A: Pyrethroid	Adult
λ-cyhalothrin	3A: Pyrethroid	Adult
Deltamethrin	3A: Pyrethroid	Adult
Clothianidin	4A: Neonicotinoid	Larva
Dinotefuran	4A: Neonicotinoid	Larva
Imidacloprid	4A: Neonicotinoid	Larva
Spinosad	5: Spinosyn	Larva
Indoxacarb	22A: Oxadiazine	Larva
Chlorantraniliprole	28: Diamide	Larva
Cyantraniliprole	28: Diamide	Larva

Chapter 2. Distribution of Annual Bluegrass Weevil in Virginia

Abstract

Annual bluegrass weevil (*Listronotus maculicollis* Dietz) (Coleoptera: Curculionidae) (ABW) is the most damaging insect pest of cool-season golf course turfgrasses, most notably annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.) in the northeastern US. Until the mid-2000s, ABW was believed to be concentrated in the northeastern region of the U.S. but has since spread south into North Carolina, west into Ohio, and north into Ontario, Canada. The western distribution of ABW has since expanded to include Kentucky (Anonymous 2021). The overall establishment and damage caused by ABW across Virginia golf courses is constantly evolving. Based on a survey conducted by McGraw and Koppenhöfer (2017) of golf course superintendents in the northeastern states, thirty from Virginia reported ABW to be present on their golf courses. In 2019, a survey was conducted in order to confirm and further understand the spread of ABW in Virginia. Two hundred and three golf course superintendents in Virginia were contacted with regards to whether they had a confirmed case of ABW at their golf course. Based on 50 responses, 34 golf courses (68%) responded with confirmed cases of ABW, leaving 16 golf courses unaffected (32%). Fifteen of the 16 courses that reported not having ABW also have bermudagrass (*Cynodon dactylon*) fairways, which are not suitable for weevil development. These data strongly suggest that ABW is well established in Virginia and continues to spread.

Introduction

Annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is the most damaging pest of cool-season golf course turfgrass in the northeastern United States (McGraw and Koppenhöfer 2017). While this weevil prefers to oviposit, develop, and feed on annual bluegrass (*Poa annua* L.), it can also develop on creeping bentgrass (*Agrostis stolonifera* L.) (Koppenhöfer et al. 2018). Annual bluegrass weevil is particularly damaging to short-mown areas of golf courses including tees, fairways, collars, and greens (McGraw and Koppenhöfer 2008).

Annual bluegrass weevil was first reported in Connecticut in 1931 (Britton 1931) and, until the 1990s, was concentrated around the metropolitan area of New York, including New Jersey and Connecticut (McGraw and Koppenhöfer 2008, McGraw and Koppenhöfer 2017). The pest has consistently expanded its distribution and range and has now spread to Quebec and Ontario in the north, western North Carolina in the south, and as far west as Kentucky in the United States (Billeisen 2017, McGraw and Koppenhöfer 2017, Anonymous 2021). Annual bluegrass weevil has one to four generations per year depending upon the environmental conditions within geographic location (McDonald and Dernoeden 2007), with more generations the further south the location is. In the Northeast US, adults emerge from overwintering during warm periods from late March to early May. Emergence is often linked to plant phenological indicators including forsythia (*Forsythia* spp.) 50% petal loss, dogwood (*Cornus* sp.) full bloom, and eastern redbud (*Cercis canadensis*) (Tashiro 1987, Diaz and Peck 2007, McGraw et al. 2020). In the Mid-Atlantic region, adult ABW become active in late February to mid-March depending on the location (Billeisen 2017). Once overwintered adults have emerged, they can quickly cycle through their generations and, often, multiple life stages are present

simultaneously. Because of ABW's complex life style, management of the pest has become one of the most expensive insect pest problems the golf industry has ever seen (McGraw and Koppenhöfer 2017).

While the majority of ABW research has been conducted in the northeastern United States, little is known about ABW as it pertains to Virginia. Based on the review of the literature, there have been no Virginia-specific distribution studies or maps created to explore and give insight to the status of ABW as it currently stands in Virginia. The objective of our research was to determine the distribution of ABW in Virginia and give further insight as to the kinds of golf courses affected by conducting a survey of golf course superintendents inquiring about the presence or absence of ABW on their course, the confirmation of their presence by a turfgrass professional, and the grasses grown on their course in the areas most affected by ABW: putting greens, fairways, tee boxes, and collars.

Materials and Methods

Survey Form and Population

This survey was conducted throughout 2019 and it asked if ABW was present on the golf course, if the presence of ABW had been confirmed by an industry representative, extension specialist, entomology lab, or university professional, and the types of turfgrasses grown on the course's putting greens, collars, tee boxes, and fairways. All survey questions were open ended, allowing the responding party to answer with as much or as little detail as they saw fit. The survey was sent via email individually and did not utilize a survey website or survey software, so no external links or websites were required to respond to the survey questions. If any questions were left unanswered in the response, the superintendents were sent a follow-up email to clarify the unanswered questions.

Using the Virginia Chapter Golf Course Superintendent's Association Membership Directory (2019), a list of golf course superintendents was compiled including their name, email, and golf course. The list was then cross-checked for multiple people tied to the same golf course and only one primary contact (usually the superintendent or Director of Agronomy) was contacted from each facility to minimize the chance of multiple responses from the same golf course.

Data Collection

All survey responses were manually recorded in an Excel (Microsoft Corporation, Redmond, WA) spreadsheet and separated into two initial groups: ABW positive or ABW negative. Within the positive group, responses were then further separated into a "confirmed" group or an "unconfirmed" group. While all responses were valuable, this further separation into confirmed and unconfirmed groups limited the possibility of false-positive data being interpreted as positive ABW. Within each group, grass species was recorded but did not include cultivars, as not every response included such.

Results

Two-hundred and three golf course superintendents in Virginia were contacted and 50 responses were attained (24.6%). Based on 50 responses, 34 golf courses (68%) responded with confirmed cases of ABW (Fig. 2.1), leaving 16 courses unaffected (32%). Fifteen of the 16 courses that reported not having ABW also have bermudagrass (*Cynodon dactylon* L.) fairways, however 14 of the 16 courses have cool-season putting greens. There was only one course that reported no ABW that was grassed entirely in cool-season turfgrasses. Of the 16 courses that reported no ABW, nine of them were east of Richmond, with eight of those nine being in the Coastal Plain/Tidewater region of Virginia. The other seven courses that reported no ABW were

west of Richmond in the Piedmont, Blue Ridge, and Valley and Ridge regions of Virginia. The only fully cool-season turfgrass golf course that reported no ABW were located in the mountainous region of southwestern Virginia, 12 km north of the Virginia-North Carolina border. Of the 34 confirmed positive ABW courses, seven expressed that ABW have been present on their course for four seasons or less (20.6%), as of the summer of 2019. The seven golf courses experiencing ABW pressure for four seasons or less are in Spotsylvania, Triangle, Amherst, Manakin-Sabot, Basye, Harrisonburg, and Luray (Fig. 2.2)

Discussion

My survey response data suggest that ABW is well-established on cool-season turfgrass golf courses in Virginia as previously suggested by the survey of McGraw and Koppenhöfer (2017). This survey recorded sixteen positive ABW infestations in Virginia, compared to fourteen positive ABW infestations recorded by McGraw and Koppenhöfer (2017), and provided insight into golf courses that reported no ABW presence. Fifteen of the sixteen ABW negative golf courses reported *C. dactylon* fairways, a warm-season turfgrass unfavorable thus far to ABW oviposition and development. However, fourteen of the sixteen ABW negative courses also reported cool-season turfgrass putting greens, with ten of these course putting greens consisting of only *A. stolonifera* and the other four courses consisting of mixed *A. stolonifera* and *P. annua* putting greens. Fairways, on average, make up the second largest majority of turfgrass acreage on most golf courses at 28 acres per 18-hole golf course, while putting greens account for an average of only 3.2 acres (GCSAA 2017). These initial observations suggest that while ABW can oviposit and develop on *A. stolonifera* (Kostromytska and Koppenhöfer 2014), golf courses that utilize warm season fairways with *A. stolonifera* putting greens do not provide sufficient acreage nor favorable conditions for ABW to survive. The four ABW negative golf

courses with mixed *A. stolonifera* and *P. annua* putting greens but *C. dactylon* fairways, while more favorable for oviposition than a pure *A. stolonifera* turfgrass stand (Kostromytska and Koppenhöfer 2014), likely does not provide enough acreage to support ABW populations.

Of the thirty-four confirmed positive ABW infestations, ten (29.4%) utilized warm-season turfgrass fairways, consisting of either *C. dactylon* or zoysiagrass (*Zoysia* spp.). These results contradict the previous assumptions that a golf course limited to cool-season putting greens with warm-season fairways would not be suitable enough for the development and survival of an ABW population. The cause of this variability between present ABW populations is unknown. To determine the cause of select golf courses with cool-season putting greens and warm-season fairways being suitable for ABW populations while others remain unaffected would require more detailed accounts of ABW presence on the affected courses, i.e., when the weevils first appeared, what area they appeared on initially, where the population originated from, etc.

Of the seven golf courses reporting ABW presence for four seasons or less, three have warm-season turfgrass fairways with one of these three being a full *C. dactylon* golf course but with *A. stolonifera* putting greens. This suggests that *A. stolonifera*, the less favorable ABW-affected grass, putting greens alone are enough to support an ABW population, a notion that contradicts the initial assumptions of this survey that cool-season putting greens alone are unable to support an ABW population.

Based on the survey results, ABW is widespread across Virginia with few unreported areas. Annual bluegrass weevil infestations are occurring further south and west than previously reported, validating the continued spread to new regions. While golf courses with a majority of cool-season turfgrasses are most affected, we suggest that golf courses with warm-season

fairways and annual bluegrass and/or creeping bentgrass putting greens continue to monitor for ABW, as our data shows that weevil survival is possible under such scenarios.

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Figures

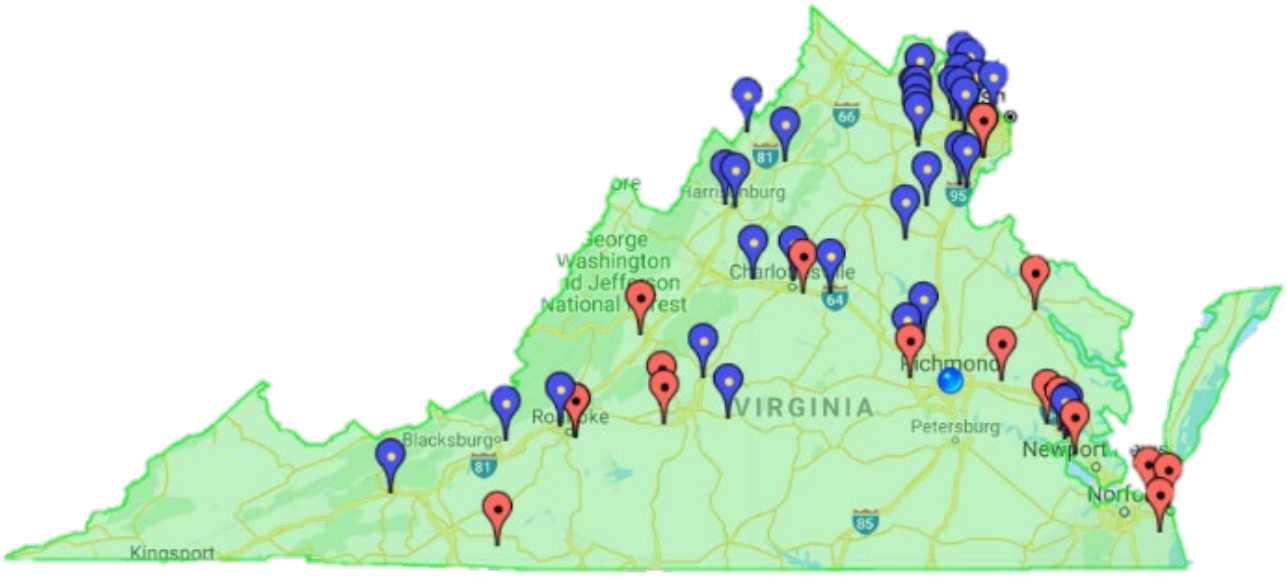

Figure 2.1. Distribution of annual bluegrass weevil (ABW) in Virginia based on a 2019 survey of golf courses. The darker/blue icons represent survey respondents that indicated ABW presence has been confirmed. The light/red icons indicate locations where ABW has not been found.

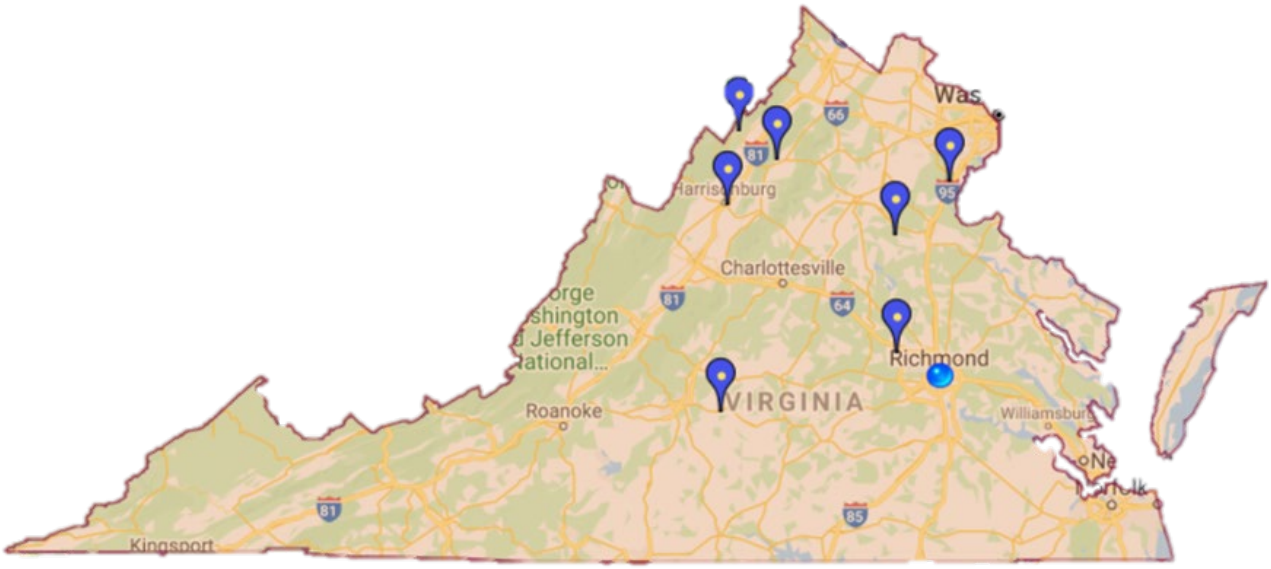

Figure 2.2. Locations of golf courses in Virginia reporting annual bluegrass weevil presence for four seasons or less, as of the summer of 2019.

Chapter 3. Seasonal Biology of Annual Bluegrass Weevil

Abstract

Annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is the most damaging insect pest of cool-season golf course turfgrasses in the northeast and Mid-Atlantic United States. While the seasonal biology and phenology of ABW is well established in the northeastern US, it has not been documented in the most southern areas of its expanded distribution, where timing of ABW emergence, oviposition, and number of generations may be different from what is documented for the northern states. A study was conducted on two Virginia golf courses, in Blacksburg, VA and Roanoke, VA, in 2019 and 2020 to track the population densities of ABW adults and larvae throughout the active season. Weevils were sampled on a weekly basis and the numbers of adults, small larvae, and large larvae were plotted over time. Our data suggest that the use of GDD to track and predict the emergence of overwintered adults and subsequent life stage peaks in Virginia is less reliable than previous reports in the northeastern US. Emergence of overwintered adults occurred similarly at both golf courses in 2019 and 2020, with first adult emergence beginning in late February to early March. In both years and locations, the peak of early instars (small larvae) was observed between 01 May and 15 May, suggesting a larval application timed for early May would often be sufficient to optimize a larvicide application. Based on the data obtained on the last sampling day of each year and anecdotal evidence from golf course superintendents, it is probable that both adult and larval ABW activity persists well into late September and possible early-mid October. Four ABW adult peaks were observed at each location in both years of the study, indicating the three generations of ABW occur in western Virginia. These data provide valuable information for timing insecticide applications for ABW adults during March and targeting larvae during early

May in western Virginia, which is quite a bit earlier than currently recommended for northeastern golf courses.

Introduction

Annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is a well-established and damaging pest of cool-season turfgrass in the northeastern US, Mid-Atlantic United States, and parts of Canada (McGraw and Koppenhöfer 2017). While ABW was first reported as a pest in Connecticut in 1931 (Britton 1931), and has since been spreading southward into North Carolina (Billeisen 2017) and also westward into Ohio and Kentucky (Anonymous 2021). Effective control of ABW has been difficult due to poor timing of insecticide sprays resulting in multiple applications, resistance development to certain insecticides, and the need for different insecticides to target adults versus larvae (Kostromytska et al. 2015, McGraw and Koppenhöfer 2017, Koppenhöfer et al. 2018). Knowledge of the seasonal biology of ABW in a region is vital to proper pest management timing. While ABW is becoming increasingly common on golf courses that utilize cool-season turfgrasses (McGraw and Koppenhöfer 2017), much of what has been reported is focused on ABW in the northeastern U.S. In New York, ABW adults begin to emerge from overwintering as early as late March and continue to emerge until late April or early May, with the first generation of larvae being observable in mid-May (Vittum and Tashiro 1987, McGraw et al. 2020). In North Carolina, Billeisen (2017) reported emergence of adults in late March to early April, with larvae being present in mid-early April. The overwintering sites of adult ABW are variable from course to course, but include leaf litter, taller grass including roughs or natural grass areas, and tree lines (Vittum 1981, Diaz and Peck 2007, McGraw et al. 2020). The emergence and activity of overwintering adults has historically been correlated with plant phenological indicators including 50% petal loss in forsythia (*Forsythia* spp.), full bloom in Dogwood (*Cornus* sp.), and full bloom in eastern redbud (*Cercis canadensis*) (Diaz and Peck 2007, McGraw and Koppenhöfer 2017, McGraw et al. 2020). While phenological indicators are helpful, they can be variable between

plant cultivars and whether plants are in full sun or not (Coustham et al. 2012). In New York specifically, overwintering emergence is reported to occur roughly between 110-120 growing degree days (GDD) with a base of 50°F beginning March 1 with these overwintering adults laying eggs at ~175 GDD (base 50°F) (Rossi 2019). However, scouting for ABW life stages is the most reliable way of determining overwintering emergence dates. After adult weevils have emerged and eggs have been deposited, the eggs laid hatch in 4-5 days (Peck et al. 2007). Once hatched, the larvae burrow down inside the plant, consuming plant material as they grow. The larvae will go through five instars, with instars 1-4 lasting 5-7 days each (Billeisen 2017), and the fifth and final instar lasting 2-5 days (Peck et al. 2007). In New York, the transition from third to fourth instar, timing considered important for proper larvicide application, is reported to coincide with ~350 GDD (base 50°F) (Rossi 2019). However, the reported number of GDD each life stage occurs at is highly variable between sources, with 700 GDD also being suggested as the proper timing for the first larval activity peak (Koppenhöfer et al. 2018). Once the fifth instar is complete, the ABW larvae forms into a pupa, with this stage lasting 6-17 days (Peck et al. 2007) after which, the ABW will emerge from the pupal cell as a teneral adult, with a soft brown exoskeleton. With maturation, the brown soft exoskeleton will develop into the hardened black-brown exoskeleton observed in mature ABW adults.

Because little is known specifically about the seasonal biology of ABW in Virginia, the objective of this study was to closely monitor their life cycle on two Virginia golf courses to gain insight into emergence and density peaks through regular, season-long sampling.

Materials and Methods

Site Description

Two golf courses in Virginia were used to monitor adult and larval populations, Blacksburg Country Club (Montgomery County VA) (37.213536, -80.359322, elevation 460.86m) (BCC) and Ballyhack Golf Club (Roanoke County VA) (37.227144, -79.890619, elevation 334.67m) (BHGC). At BCC, a fairway section (60% *P. annua*, 40% *A. stolonifera*) of approximately 250 m^2 was left untreated with insecticides and was used for sampling. At BHGC, a fairway target (~475 m^2) on the driving range (5% *P. annua*, 95% *A. stolonifera*) that was not treated with insecticides was used. This driving range target was chosen for its proximity to higher cut turfgrass areas, where ABW can overwinter. In 2019, ABW were sampled weekly from 28 Feb to 29 Aug at both locations. In 2020, weevils were sampled weekly from 3 Mar to 10 Sep at BHGC and 3 Mar 2020 to 17 Sep 2020 at BCC.

Adult Sampling

Adult ABW were sampled using the soap flushing method following Koppenhöfer et al. (2020). Lemon scented liquid detergent dish soap (Ajax Ultra Super Degreaser Lemon Dish Liquid) was used as the irritant at a rate of 7.8 mL soap L^{-1} water. The soapy water mixture was initially applied to the turfgrass at a rate of 1 L mixture per 0.092 m^2 (= 1 ft^2) turfgrass. The areas of soap flushing were chosen at random each week from within the previously described sampling area. After the initial soapy water mixture was applied to the turfgrass, the surface of the grass was searched for ABW adults until no weevils had been counted for two minutes. After two minutes of observing no weevils, the collection area was then re-soaked with 1 L of soapy water mixture. Any additional weevil adults were collected after this second soaking for 5-10 minutes. At each weekly sampling, a total of three 0.092 m^2 samples were soap flushed, and the number of ABW adults was averaged using those three counts.

Larval Collection

Densities of ABW larvae were estimated before or after the adult soap flushing on the same collection day from adjacent sampling locations weekly using a 5-cm diameter soil sampling probe at three random locations within the untreated sampling area. After soil core plugs were collected, they were immediately transported from the golf courses to the lab in sealed 0.9-liter plastic containers. Soil core plugs were trimmed to a depth of 3-cm below the thatch layer where larvae would be found (Beard 2002). The plugs were then broken apart thoroughly by hand and placed back into their plastic container where a saltwater solution of 119g NaCl L^{-1} water was poured over the broken plugs and the container was filled to 1.5-cm over the soil core layer. The container was then sealed and shaken vigorously by hand for two minutes to dislodge any larvae within the grass plants, thatch, and soil. The container was immediately examined for larvae. After the initial examination was complete, the container was covered and left to sit for one hour. After one hour, the solution was stirred and re-agitated briefly then examined again for any additional larvae to determine the final larval density separated into small larvae (instars 1-3) and large larvae (instars 4-5).

Temperature/Degree-day Recording

In each year, daily max-min temperature data were collected at each sample site using a WatchDog 2000 Series Mini Station data loggers (Spectrum Technologies, Inc., Aurora, IL). The loggers are completely waterproof and feature 12-bit resolution. Temperature data were used to calculate growing degree-days (GDD) beginning March 1 ([maximum daily temperature (ºF) + minimum daily temperature]/2 - 50 ºF). The Fahrenheit scale was used for data recording as it is the standard for calculating GDD in the U.S. golf industry and made communications and comparisons with past data less confusing.

Results and Discussion

In Blacksburg, the adult ABW densities peaked at 48 weevils per 0.092 m^2 (1 ft^2) in 2019 and 53.7 per 0.092 m^2 in 2020 (Tables 3.1, 3.2). Annual bluegrass weevil larval bodies were sampled beginning in the second week of April in 2019 and the third week of April in 2020. The number of small larval bodies 0.092 m^{-2} peaked at 54.4 in 2019 and 136 in 2020, with large larval bodies 0.092 m^{-2} peaking at 136 in 2019 and 122.4 in 2020. While small larval counts increased from 2019 to 2020, large larval body counts were similar between the years. Experience with identifying earlier instar larval bodies in the salt floatation solution could account for this increase. Later instar larval bodies are relatively easy to identify in the floatation solution compared to earlier instars, which could account for the similar ranges in larger larval bodies between 2019 and 2020.

At BHGC, the number of ABW adults 0.092 m^{-2} was similar between sampling years with densities peaking at 40.66 in 2019 and 43.33 in 2020 (Tables 3.3, 3.4). The range of small larvae 0.092 m^{-2} in 2019 was 0 to 95.2 and 0 to 54.4 in 2020, showing a decrease in larval densities between the sampling years. Like the range of small larvae, the range of large larvae also decreased between 2019 and 2020, peaking at 136 0.092 m^{-2} and 81.6 0.092 m^{-2}, respectively. Unlike BCC where smaller larvae identified 0.092 m^{-2} increased from 2019 to 2020 likely due to more experience with identifying earlier instars, the decrease in both large and small larvae at BHGC could be due to an overall decrease in larval pressure and natural survivability. The sampling area at BHGC was highly saturated from rainfall on multiple assessments due to the position and slope of the sampling area relevant to the surrounding area. In 2019, rainfall in Roanoke from 01 Mar. – 01 Sep. was 1.42cm less than the average rainfall for this time frame (National Weather Service Forecast Office). During the same months in

Roanoke in 2020, rainfall was 35.74cm more than the average rainfall (National Weather Service Forecast Office). This relatively large increase in rainfall combined with the position of the sampling area could be a possible cause of the decrease in larval body counts from 2019 to 2020 from BHGC, as BCC also experienced an increase of rainfall 27.28cm more than the average but is not situated downhill from a severe slope as the BHGC sampling area is.

Both BCC and BHGC observed four ABW adult peaks in 2019 and 2020 (Figs. 3.1, 3.2). However, while both sampling years exhibit four adult peaks at both locations, the dates of the adult density peaks do not coincide between years at either golf course. The adult peaks observed at BCC during 2019 were on 03 Apr (25 GDD), 14 Jun (1017 GDD), 28 Jun (1306.5 GDD), and 23 Jul (1954.5 GDD) (Figs. 3.1a, 3.3a) while in 2020 the peaks were observed on 23 Apr (184.5 GDD), 11 Jun (749 GDD), 04 Aug (2007.5 GDD), and 18 Aug (2327 GDD) (Figs. 3.1b, 3.3a). At BHGC, the adult peaks observed during 2019 fall on 23 Apr (292 GDD), 07 Jun (1228 GDD), 27 Jun (1686.5 GDD), and 31 Jul (2675.5 GDD) (Figs. 3.2a, 3.3b) which is relatively similar to the adult peak dates from BCC in the same year. In 2020, the adult peaks occurred on 25 Mar (113 GDD), 01 May (390.5 GDD), 23 Jun (1302 GDD), 18 Aug (2953 GDD) (Figs. 3.2b, 3.3b). Unlike 2019, these adult peaks are not as similar to the 2020 adult peaks at BCC. Although four adult peaks can be seen, the first peak likely consists only of emerging overwintered adults, showing three generations of ABW. Three generations of ABW are consistent with the literature describing the ABW life cycle in more southern states including Maryland, Virginia, and North Carolina (McDonald and Dernoeden 2007, Billeisen 2017). Similar adult activity periods have been observed on golf courses in northern Virginia by golf course pest management consultants Steve McDonald and Sam Camuso since 2016 (McDonald and Camuso 2021).

Larval densities varied between the two locations. In 2019 and 2020, BCC exhibits roughly four peaks of small larval bodies (2019: 15 May (475 GDD), 28 Jun (1306.5 GDD), 23 Jul (1954.5 GDD) 29 Aug (2789 GDD); 2020: 01 May (211.5 GDD), 29 May (483.5 GDD), 07 Jul (1265.5 GDD), 04 Aug (2007.5 GDD)), while large larval bodies are only seen to show three distinct peaks in each year (2019: 23 May (615 GDD), 28 Jun (130.65 GDD), 23 Jul (1954.5 GDD); 2020: 29 May (483.5 GDD), 11 Jun (749 GDD), 7 Jul (1265.5 GDD)). This suggests that sampling was not extended far enough into the fall to capture a possible fourth large larval peak. Unlike BCC, at BHGC, three peaks were observed for both small and large larval bodies. Small larval body peaks were consistent in date between 2019 and 2020 with the exception of the second larval peak (2019: 08 May (560.5 GDD), 14 Jun (1354 GDD), 13 Aug (3022 GDD); 2020: 07 May (450 GDD), 07 Jul (1704.5 GDD), 26 Aug (3171.5 GDD)). The large larval body peak dates were more consistent between sampling years with the 2019 large larvae peaks on 23 May (849.5 GDD), 07 Jul (1989.5 GDD), and 13 Aug (3022 GDD) and 2020 peak dates falling on 15 May (496 GDD), 07 Jul (1704.5 GDD), and 26 Aug (3171.5 GDD). The consistent early instar larval peaks between 01 May and 15 May at both locations during both sampling years suggests a larvicide application applied during the second week of May would be sufficient to optimize that application for larval control. This is vastly earlier than recommendations for the northeastern US, where the first generation of large larvae appear in July to early August, with a variable third generation possible in mid-August to mid-late September (Koppenhöfer et al. 2012).

While an ABW adult peak was not observed at BCC in 2020 until 23 Apr, consistent adult activity was present leading up to the first peak. Adult activity from 10 Mar to 14 Apr increased from 4.3 weevils 0.092 m^{-2} on 10 Mar to 12.66 weevils 0.092 m^{-2} on 09 Apr, with a

slight decrease to 12.5 weevils on 14 Apr, the sampling day before the first adult peak on 23 Apr. At BHGC in 2019, a similar late overwintering adult peak was observed on 23 Apr, but consistent adult activity was also present leading up to the peak from 14 Mar to 17 Apr with 7 weevils ft^{-2} to 10.6 weevils ft^{-2}. The largest adult peak in 2020 was observed on the same sampling date for both locations, 53.67 weevils 0.092 m^{-2} on 18 Aug at BCC and 43.33 weevils 0.092 m^{-2} on 18 Aug at BHGC. These large adult peaks do not occur late in the season in the northeast as they appear to in Virginia. This increase in adult activity later in the season could suggest higher densities of overwintering adults than observed in the northeast.

Based on the ABW population density data collected in the 2019 and 2020 seasons, adults emerge from overwintering in southwestern Virginia in late February or very early March, with most first adult peaks (BCC 2019 and 2020, BHGC 2019) occurring in April. It is likely that adult and larval ABW activity continues into September and October based on the data retrieved on the last sampling day of each year, 29 Aug in 2019 and 10 Sep at BHGC in 2020 and 17 Sep at BCC in 2020 as well as anecdotal evidence from golf course superintendents. Continuance of ABW pressure well into the fall and early winter does not occur in the northeast. Sampling for population densities should continue well into the fall until adult and larval activity has ceased for several sampling days in order to determine if more generations occur in Virginia. Growing degree days, both cumulative and between adult peaks, did not show any distinct trends. Growing degree day calculation began on 1 Mar, or 28 Feb, dependent on first sampling date. At BCC, the number of GDD accumulated between adult peaks in 2019 ranged from 289.5 to 992 and at BHGC, ranged from 458.5 to 992. In 2020, at BCC, the number of GDD accumulated between adult peaks ranged from 319.5 to 1258.5 and at BHGC, ranged from 277.5 to 1651. In 2020, the low end of each range varied between courses, with 319.5 GDD

accumulating between peaks at BCC in Aug and 277.5 GDD accumulating between peaks at BHGC in Apr. The first appearance of larvae and subsequent GDD accumulated also varies between courses and years. In 2019 at BCC, larvae first appeared at 475 cumulative GDD and at 211.5 GDD in 2020. At BHGC, the gap between years was not as severe as the gap between years at BCC. In 2019, larvae were first found at 560.5 GDD and 450 GDD in 2020. At BCC, between the May and June larval peaks in 2019, 831.5 GDD accumulated, between the June and July peaks, 648 GDD accumulated, and between the July and August peaks, 834.5 GDD accumulated. In 2020, 272 GDD accumulated between the first and second larval peaks, 782 GDD between the second and third peaks, and 742 between the third and fourth peaks. At BHGC in 2019, between the first small larval peak in May to the second peak in June, 793.5 GDD accumulated and 1668 GDD accumulated between the second peak and the third peak in August. In 2020, 1254.5 GDD accumulated between the first peak in May and the second peak in July. Between the second peak and the third peak in August 1467 GDD accumulated. The GDD accumulation between large larval peaks at BCC varied between the years with a range of 265.5 GDD to 691.5 GDD. The variability was not as extreme at BHGC between years, with a range of 1032.5 GDD to 1467 GDD. In the northeast, the first peak of late instar larvae based on GDD is variable with some reports suggesting this occurs at 700 GDD (Koppenhöfer et al. 2018) while others report 350 GDD for the same life stage (Rossi 2019). However, our first late instar larval peaks occurred at 483.5 GDD, 496 GDD, 615 GDD, and 849.5 GDD, showing variability between courses and years that does not adhere to the 700 GDD peak or 350 GDD reported in the northeast. Taking into consideration all GDD data across the life stages sampled for, there does not seem to be any immediate trends. To further explore GDD in relation to ABW activity

in Virginia and determine trends between GDD and activity, courses could be sampled more often than weekly to obtain more data.

While the use of GDD calculations to predict the life cycle of ABW is common in the northeast, resources available report variable information. In one instance, the first large larval peak of ABW occurs at 700 GDD (base 50°F, beginning March 1) (Koppenhöfer et al. 2018), while another source describes the transition from small earlier instar larvae to larger later instar larvae occurring at 350 GDD (Rossi 2019). Our GDD data provided variable information between courses and years, showing no trends or patterns in overwintering emergence, larval activity, or adult activity. Despite the variance in GDD data, the calendar dates of emergence and larval activity were consistent between years and locations. However, over a longer period of time and more sampling years, this consistency may not hold true, as weather is often variable between years. Our population density data reveals the occurrence of three clear ABW generations, with a probable fourth generation. Annual bluegrass weevil activity likely continues throughout the fall and into early winter based on anecdotal evidence, however, continued sampling into the fall and winter would be required to confirm this. Adult peaks occurring later in the season, such as the peak on 18 Aug at both locations in 2020, are not consistent with the peaks observed in the northeast, which happen earlier in the season. This increased activity during the later parts of the season could lead to an increase in the number of adults that overwinter and subsequently emerge the next spring. However, the relationship between the abundance of ABW adults going into overwintering and the timing of overwintering emergence has not been researched.

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Figures

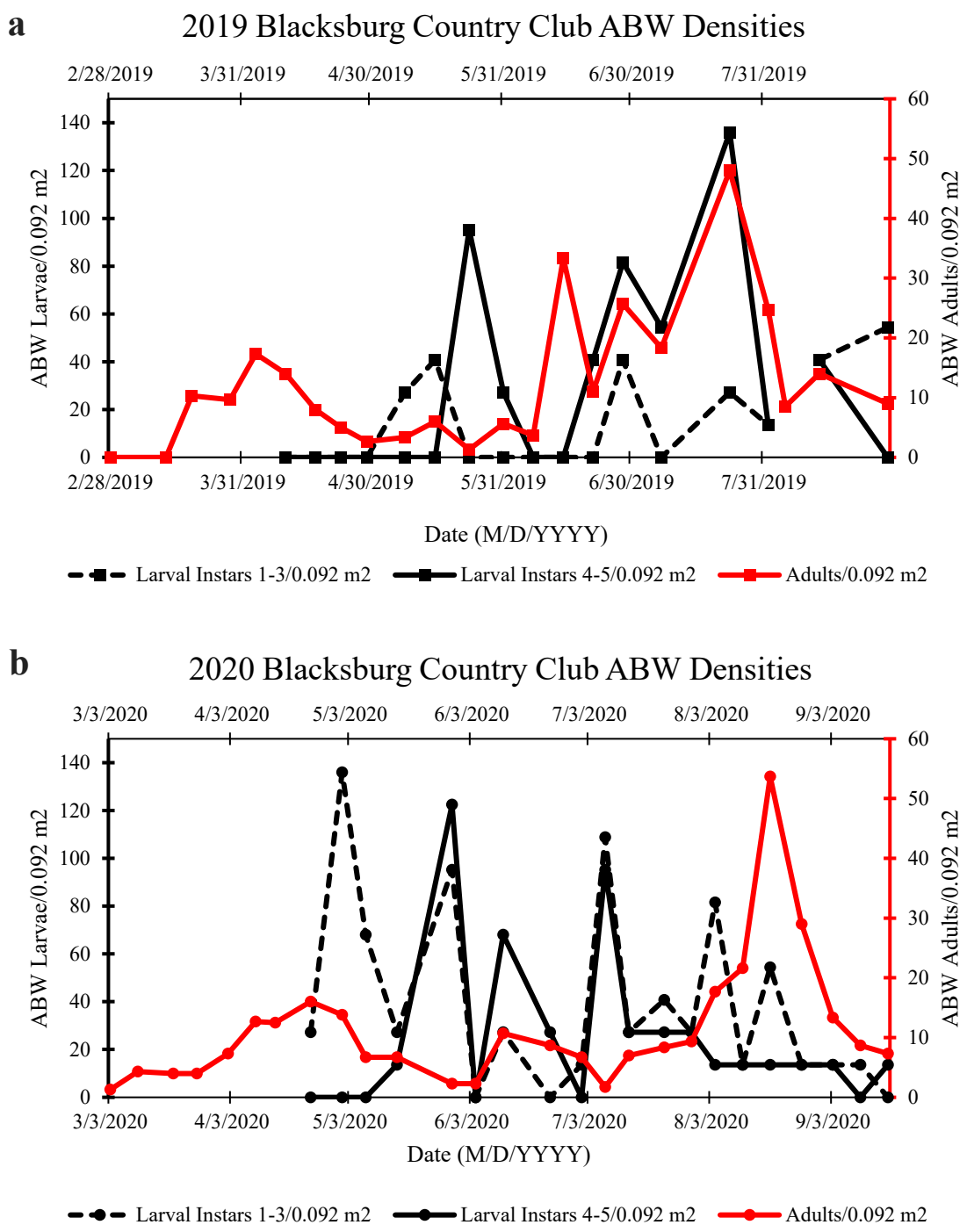


Figure 3.1. Annual bluegrass weeivil (ABW) population densities from Blacksburg Country Club (Montgomery County VA) during a) the 2019 season and b) the 2020 season. The dashed black line indicates small larvae, larval instars 1-3, the solid black line indicates large larvae, larval instars 4-5. Both larval densities are graphed in accordance with the left y-axis. The

lighter/red solid line indicates the adult densities and is graphed in accordance with the right y-axis.

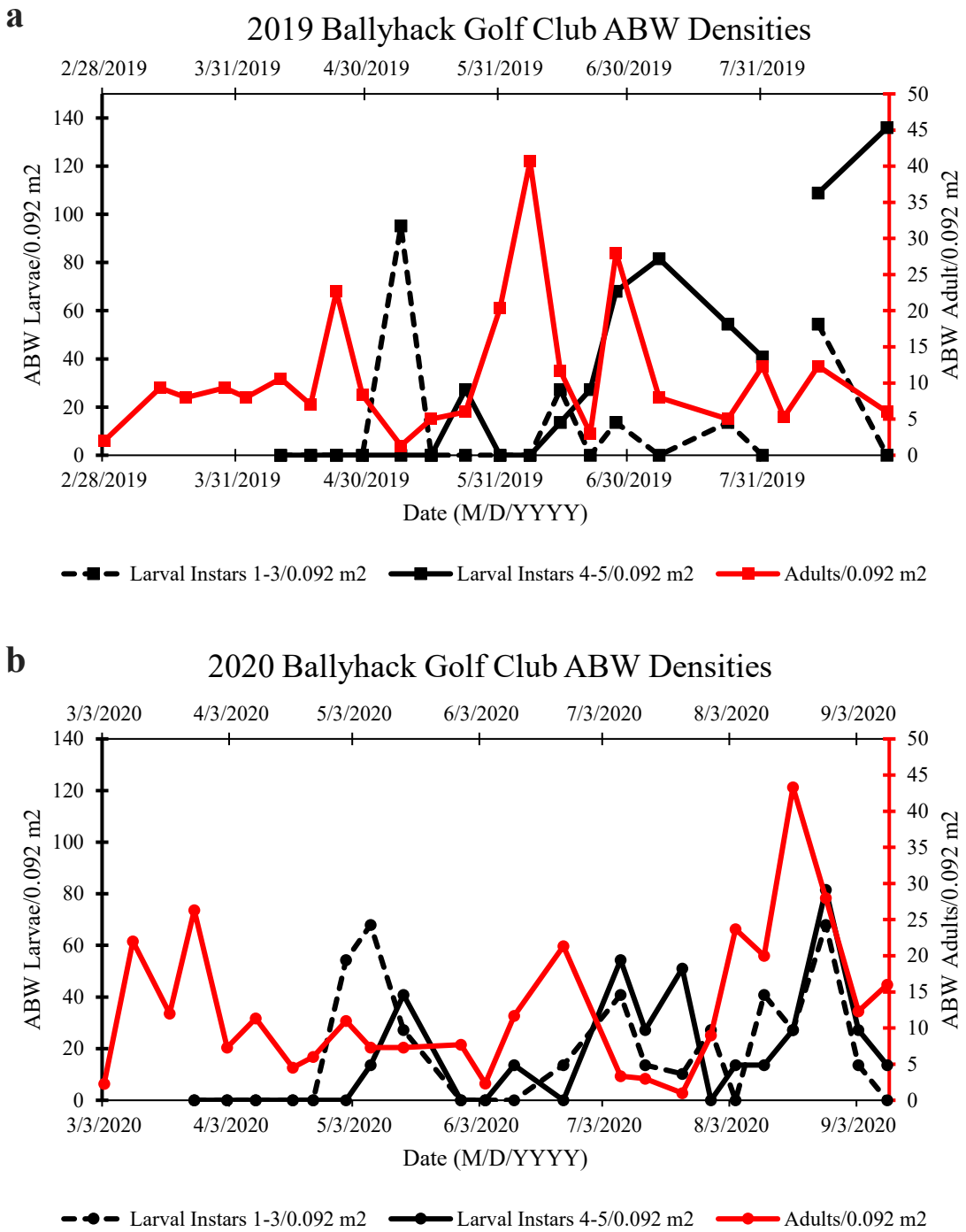
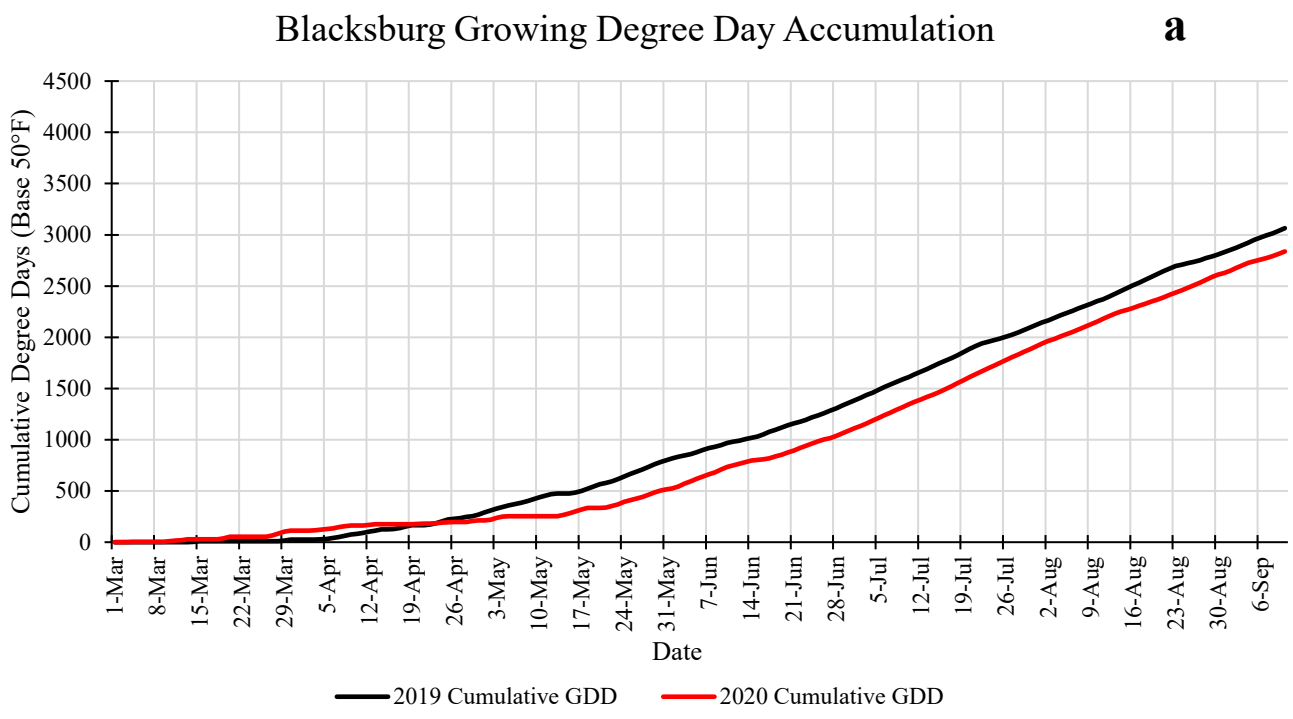


Figure 3.2. Annual bluegrass weevil (ABW) population densities from Ballyhack Golf Club (Roanoke County VA) during a) the 2019 season and b) the 2020 season. The dashed black line indicates small larvae, larval instars 1-3, the solid black line indicates large larvae, larval instars 4-5. Both larval densities are graphed in accordance with the left y-axis. The lighter/red solid line indicates the adult densities and is graphed in accordance with the right y-axis.



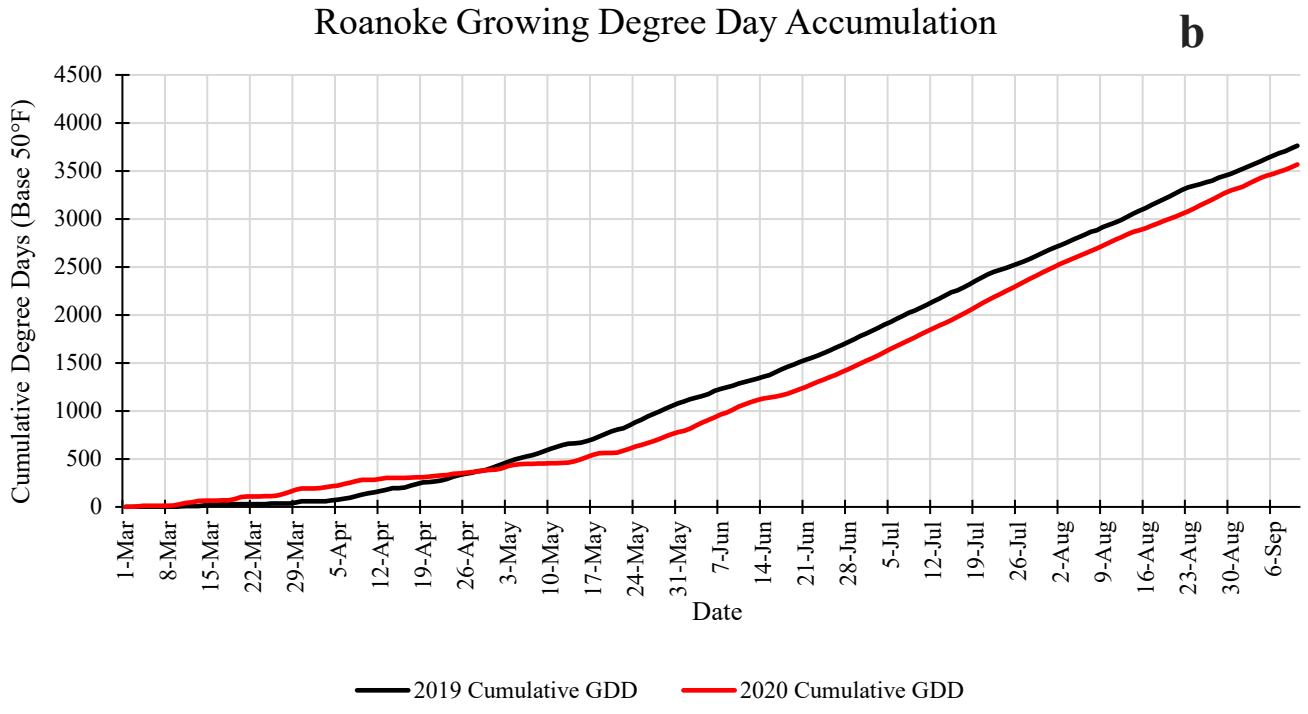


Figure 3.3. Growing degree day (GDD) accumulation (base 50°F) from 2019 and 2020 in a) Blacksburg, Virginia (Montgomery County) and b) Roanoke, Virginia (Roanoke County). The 2019 cumulative degree day trend is in black, and the 2020 trend is in red/lighter color.

Chapter 4. Comparison of Two Annual Bluegrass Weevil Larvae Extraction Techniques

Abstract

Annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is an established pest of cool-season golf course turfgrasses in the northeastern and Mid-Atlantic US, most notably annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.). Scouting for ABW adults and larvae is essential for proper application timing. Current recommendations for extraction and sampling ABW larvae involve collecting soil plugs, breaking them apart in a saltwater solution, and counting the number of larvae that float to the surface. Noticing small larvae and sifting through numerous tiny grass clippings on the surface are drawbacks of this method. Use of heat extraction has been explored recently as an alternative to salt floats. The technique uses a heat lamp to dry the soil plug in a Berlese-Tullgren funnel and force ABW larvae downward though the soil and into a catch jar in order to escape the heat. Our research objective was to compare these two extraction methods using soil plugs collected from two different golf courses in Virginia with established populations of ABW. Larvae from matched pair plant and soil cores were extracted using either the salt float method or the Berlese-Tullgren funnel method. Results showed that the two extraction methods are highly correlated with regards to the number of ABW larvae collected ($r^2 = 0.7955$), and there was no significant difference between the methods in number of larvae extracted ($P = 0.408$). Our research suggests that both methods are comparable for extracting ABW larvae and that the method used can be determined by the turfgrass manager based on external factors such as time, experience, and preference.

Introduction

Annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby, is the most damaging pest of cool-season golf course turfgrass in the northeastern and mid-Atlantic United States (McGraw and Koppenhöfer 2017). Annual bluegrass weevil is particularly damaging to areas of golf courses that are short-mown including tee boxes, putting greens, fairways, and collars (Fig. 1.1). To mitigate this damage, the use of chemical insecticides is the most common and effective way to control and treat for ABW (Vittum 1999). However, doing so is met with several challenges. The first challenge is the differing insecticide classes and active ingredients used to treat the adult and larval stages of ABW (Table 1.1). The most common insecticide classes used to treat the adult stage are pyrethroids including bifenthrin, λ-cyhalothrin, deltamethrin and the organophosphate chlorpyrifos (McGraw and Koppenhöfer 2017). The larval stage of ABW are typically treated with anthranilic diamides such as chlorantraniliprole or cyantraniliprole, neonicotinoids such as imidacloprid, or other insecticides like trichlorfon, spinosad, or indoxacarb (McGraw and Koppenhöfer 2017). The second challenge when treating for ABW is the pest's asynchronous life cycle (Billeisen 2017). At any given point during the active summer season, multiple life stages of ABW can be present (Fig. 3.1, 3.2), including eggs, larvae, pupae, and adults. These two factors make choosing the accurate insecticides and proper application timing challenging for golf course superintendents.

In the Mid-Atlantic region, adult ABW become active in late February to mid-March dependent on both locational and environmental conditions (Billeisen 2017). Historically, emergence of overwintering adults has been correlated to plant phenological indicators including forsythia (*Forsythia* sp.) at 50% petal loss, or dogwood (*Cornus* sp.) and eastern redbud (*Cercis canadensis* L.) at full bloom (Tashiro 1987, Diaz and Peck 2007, McGraw et al. 2020). However,

plant phenological indicators often are not consistent with the industry recommendations of application timing (Vittum and Brocklesby 2013) and vary between cultivars and even on the same cultivars on the same golf course (McGraw et al. 2020). The use of plant phenological indicators is considered helpful for timing ABW treatment but should not be the only factor used for proper application timing. Female ABW begin oviposition in the spring, with one study reporting that three weeks after emergence, all females collected possessed well-developed reproductive systems (McGraw et al. 2020). The female adult ABW deposits two to six eggs inside of the turfgrass plant sheath (McGraw and Koppenhöfer 2008). After a few days, eggs hatch into larvae and feed on the inside of the grass plant while moving downwards toward the crown of the plant during the early instars (Diaz and Peck 2007). Once the crown of the plant is consumed during the later instars, either partially or completely, the larvae burrow down into the soil-thatch interface to continue to feed on the turfgrass roots until they are ready to pupate (Beard 2002, Diaz and Peck 2007). Annual bluegrass weevil development from egg to adult takes approximately two months, with each larval instar lasting five to seven days (Vittum 1999, McDonald and Dernoeden 2007). This, combined with the ability of the ABW adult female to lay multiple broods of offspring, allows ABW to express multiple life stages simultaneously during the active season. This makes treating for ABW challenging as different insecticides are used for different life stages.

Sampling fairways, tee boxes, and collars for ABW adults and larvae provides valuable information for deciding whether to treat and which ABW life stage to treat for. Sampling and extracting adults from the thatch and soil layers is relatively simple and can be done using one of two methods: the irritant sampling method or the vacuum sampling method. Irritant sampling was originally used to target soil-dwelling Lepidopteran larvae by drenching low doses of

pyrethrin, but has since been modified with liquid detergent as a replacement (Tashiro 1987). In one instance, a mixture with a detergent concentration of 1-2% and 10-15 minutes allotted for collection is recommended (McGraw and Koppenhöfer 2008). In a more recent study, using a mix that was 0.8% liquid detergent with a collection time of 20 minutes proved to be the most efficacious (Koppenhöfer et al. 2020).

Vacuum suction for insect sampling is often used in agricultural settings for foliage sampling (Tashiro 1987, Yi et al. 2012). There are two common types of vacuum sampling apparatuses, the Johnson-Taylor suction trap and the Dietrick vacuum insect net (D-vac) (Yi et al. 2012). Johnson-Taylor (Johnson 1950, Taylor 1951) suction traps are primarily used for catching and detaining aerial arthropods and are not considered useful for ABW sampling. The D-vac (Dietrick et al. 1960) is suited best for ground-dwelling arthropods and consists of a motorized exhaust fan and collection net. However, traditional D-vacs are considered bulky and can be expensive per unit (Yi et al. 2012, Zou et al. 2016). Because of this, modification of a traditional leaf-blower into a vacuum sampler has been used to sample for and extract ground arthropods, including ABW (McGraw and Koppenhöfer 2008, Zou et al. 2016).

While the traditional ABW adult sampling and extraction methods have been compared, the reliability of methods for larvae extraction have not been as widely studied. Extracting larvae from the turfgrass is a little more difficult as they do not come to the surface when a drench of an irritant is applied. The methods that can be used to extract larvae, the salt floatation method or the Berlese-Tullgren funnel method have not been compared (Berlese 1905, Salt and Hollick 1944, Blasdale 1974, Sotherton 1984, Hellman 1994, Yi et al. 2012, Owens and Carlton 2015, McCravy 2018, Rossi 2019). Early floatation methods utilized benzene or magnesium sulfate in combination with air and water jets to force the arthropods to the surface (Salt and Hollick

1944). Newer adaptations of the traditional floatation method substitute benzene and magnesium sulfate for common table salt (NaCl) (Sotherton 1984). The addition of table salt irritates the ABW larvae (McDonald and Dernoeden 2007) and ultimately alters the density of the water, enabling the arthropods to float to the surface for collection, without additional use of water or air jets. The salt floatation method requires removing soil cores from the desired turfgrass area, breaking the cores apart, and then submerging them in salt water for one hour (Koppenhöfer et al. 2020). This method takes relatively little active time, however, breaking apart the soil cores thoroughly without salt floatation to extract ABW larvae is also practiced (Vittum and Tashiro 1987, Ramoutar et al. 2010a).

An alternative to the salt floatation method is the Berlese-Tullgren funnel method, which utilizes heat extraction. The original Berlese funnels (Berlese 1905) used a heated water jacket as its source of heat. This heated water jacket created a flow of warm water that established a temperature gradient throughout the soil core sample. Later, Tullgren modified this funnel and replaced the water jacket with an incandescent lightbulb suspended above the soil core sample (Tullgren 1918). Tullgren's changes improved the retention and distribution of heat, established a vertical heat and moisture gradient, but ultimately increased the rate of desiccation. The later configuration, the Berlese-Tullgren funnel, is commonly used to extract micro-arthropods inhabiting the soil (Yi et al. 2012), however, in some instances (Blasdale 1974), the use of a water driven temperature gradient is preferred.

Like the salt floatation method, the Berlese-Tullgren funnel method requires soil cores of a standardized side taken from the area(s) of interest. The soil cores are then placed into a funnel where an incandescent lightbulb hanging above the funnel causes a temperature gradient (Tashiro 1987). The temperature gradient causes the arthropods in the soil core sample to move

down the sample into the collection apparatus to escape the heat source (Yi et al. 2012). This method has been used successfully for extracting billbug adults (*Sphenophorus* sp.), chinch bugs (*Blissus* sp.), webworms (*Crambus* sp.), cutworms (*Agrotis* sp.), and mites (Trombidiformes: Tetranychidae) (Niemczyk 1981). Although the Berlese-Tullgren funnel as a means of ABW larvae extraction has not been studied specifically, anecdotal evidence suggests it to be a viable means of ABW larvae extraction and sampling (McGraw 2020).

The majority of research addressing ABW extraction has been focused on the most efficacious adult life stage extraction method (McGraw and Koppenhöfer 2017, Koppenhöfer et al. 2020). To our knowledge, there have been no scientific studies comparing ABW larval extraction methods. The objective of this study was to compare the salt floatation method and the Berlese-Tullgren funnel method, to determine which is more viable for ABW larval extraction and sampling.

Materials and Methods

Soil Core Collection and Processing

Soil cores were collected from two golf courses in southwest Virginia with known ABW presence: Blacksburg Country Club (Montgomery County) (37.213536, 80.359322) (BCC) and Ballyhack Golf Club (Roanoke County) (37.227144, 79.890619) (BHGC). Soil cores were taken from both locations using a 5.08 cm core sampler. Soil core plugs were taken from a fairway (60% *P. annua*, 40% *A. stolonifera*) with a sampling area where no insecticide treatments were applied at BCC. At BHGC, a driving range target (5% *P. annua*, 95% *A. stolonifera*) left untreated with insecticides was used for core collection. Soil cores were collected nine times from BCC and six times from BHGC throughout the active summer season. Six plugs were pulled at one time from the same m^2 area, and then randomly separated into two groups of three

plugs, one group for each extraction method. Each group of three soil cores accounted for one sample group. Two sampling groups were obtained at the same time from each golf course so the extraction methods could be compared (Table 4.1). Sample groups were transported from the golf courses for 15-50 minutes in separate plastic enclosed containers and were not refrigerated due to the short transport time. A total of 18 sampling groups (54 total soil core plugs) from BCC and 12 sampling groups (36 soil core plugs) from BHGC were obtained. Soil core plugs were trimmed to a depth of 3cm below the thatch layer. Each of the sample groups taken from the same m^2 were randomly assigned an extraction method: the salt floatation method or the Berlese-Tullgren funnel method.

Salt Floatation Extraction Method

Each soil core sampling group assigned to the salt floatation method were placed in their own plastic sealing container. The plugs were broken apart thoroughly by hand and placed back into their respective plastic container. Once all three plugs from the sampling group were broken apart, the container was filled to 2.5 cm above the broken soil core layer with a saltwater solution of 119g NaCl L^{-1} in tap water. The container was then sealed and shaken vigorously by hand for two minutes. The containers were examined immediately for larval bodies. After the initial examination was complete, the containers were covered and left to sit for one hour. Once the hour was completed, the solution was re-agitated briefly and examined for larval bodies to determine the final larval count.

Berlese-Tullgren Funnel Method

The Berlese-Tullgren funnel apparatus (Fig. 4.1) used for larval extraction from soil core plugs was manufactured for this study similar to the style of the Berlese-Tullgren funnel

apparatus utilized by Sapkota et al. (Sapkota et al. 2012). Each wooden bench was constructed with the capability to house two 150-watt light bulbs. The light bulbs were suspended 25 cm above the funnel apparatus. Each soil core was housed in a 1-pint high density polyethylene (HDPE) funnel that rested on top of a glass collecting jar. To prevent clods of the soil core samples from dropping into the collecting jar, a 5cm x 5cm piece of wire mesh (5mm x 5mm) was placed inside the funnel above the lower opening (Fig. 4.2). The catch jar was filled with 20 mL of water to prevent desiccation of collected larval bodies. Each soil core from a sampling group was placed in its own funnel and all three funnels from the same sampling group were placed under one lightbulb.

Soil plug sampling groups, consisting of three soil core plugs, were broken apart thoroughly and were placed on top of the wire mesh inside of their respective funnels. All three funnels containing the broken apart soil core plugs from the sample group were arranged in a triangle (Fig. 4.1) and placed underneath of the lightbulb for 48 hours. After 48 hours, the total number of ABW larval bodies in all three catch jars were recorded as a single sampling group.

Data Analysis

Effects of extraction method, location, and their interaction were subjected to ANOVA in JMP Pro 15 (SAS Institute, Cary, NC). When appropriate, Student's *t*-test was used to separate mean differences ($P < 0.05$). The relationship between numbers of larvae extracted by the two methods was determined using linear regression in a scatterplot (Excel v2404, Microsoft 365, Microsoft Corp.).

Results and Discussion

The number of ABW larvae obtained from either extraction method ranged from 0 to 15, with the average number of larvae obtained from sampling groups being 4.4 and 3.2 for the salt floatation method and Berlese-Tullgren funnel method, respectively (Fig. 4.3). The two extraction methods tested (Table B1) were strongly correlated using simple linear regression ($r^2 = 0.7856$) (Fig. 4.4). The extraction method utilized did not have a significant effect on the number of larvae extracted ($P = 0.408$).

These results suggest that both the salt floatation extraction method and the Berlese-Tullgren funnel extraction method are comparable for ABW larvae extraction and sampling. A non-significant p-value ($P = 0.408$) suggests that the extraction method did not affect the number of ABW larvae extracted, and thus both methods obtained the same level of extraction. The extraction method utilized by a golf course superintendent in order to sample for larval bodies to correlate insecticide application timing can be decided using external factors such as time, resources, confidence in identifying larval bodies, and preference. The salt floatation method, while requiring less resources than the Berlese-Tullgren funnel method, can pose difficulty for identifying and collecting larvae; grass clippings, soil, and other debris create a crowded and busy water surface, making it difficult to immediately spot ABW larvae (Figure 4.7). The salt floatation method also requires more hands-on time in the form of shaking the sample and scanning the water for larvae. The Berlese-Tullgren funnel method requires less active time as once the plugs are broken apart, the plugs and apparatus are left for 48 hours. The small area of the catch jar and the clean water within it make identifying ABW larvae easy and relatively quick, compared to the salt floatation method. The Berlese-Tullgren funnel method requires

more advanced and planned set-up and materials than the salt floatation method, whose materials are often common household supplies.

Both the salt floatation method and the Berlese-Tullgren funnel method have advantages and minor drawbacks, however, the number of ABW larvae extracted is comparable between both. Ultimately, the golf course superintendent can decide which method is best suited for their time, materials, and ability.

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Tables and Figures

Figure 4.1. Berlese-Tullgren funnel apparatus. A 150-watt lightbulb is suspended 25 cm above the funnels. Each HDPE funnel is resting on a glass catch jar containing water to prevent desiccation of the annual bluegrass weevil larval bodies upon collection.

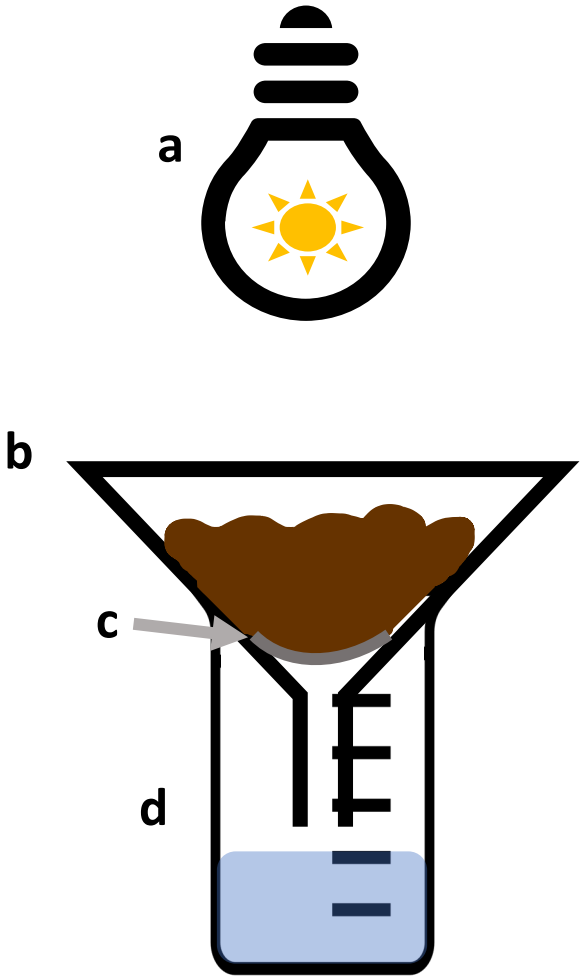

Figure 4.2. Graphic of a Berlese-Tullgren apparatus set up consisting of a) a lightbulb heat source, b) a 1-pint size HDPE funnel, c) a 5cm x 5cm piece of wire mesh, and d) a glass catch jar containing 20mL water.

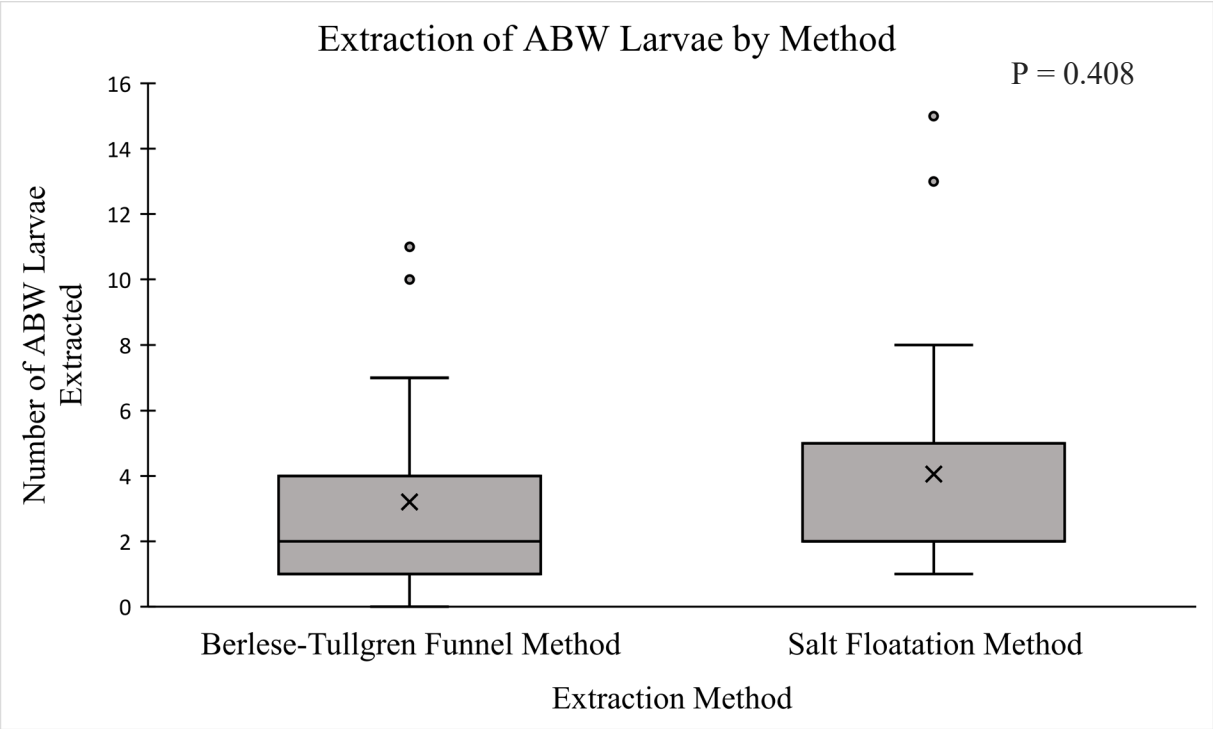

Figure 4.3. Number of annual bluegrass weevil (ABW) larvae extracted using each method. Locations are combined for each method. The extraction method used did not have a significant effect on the number of ABW larvae extracted (P = 0.408).

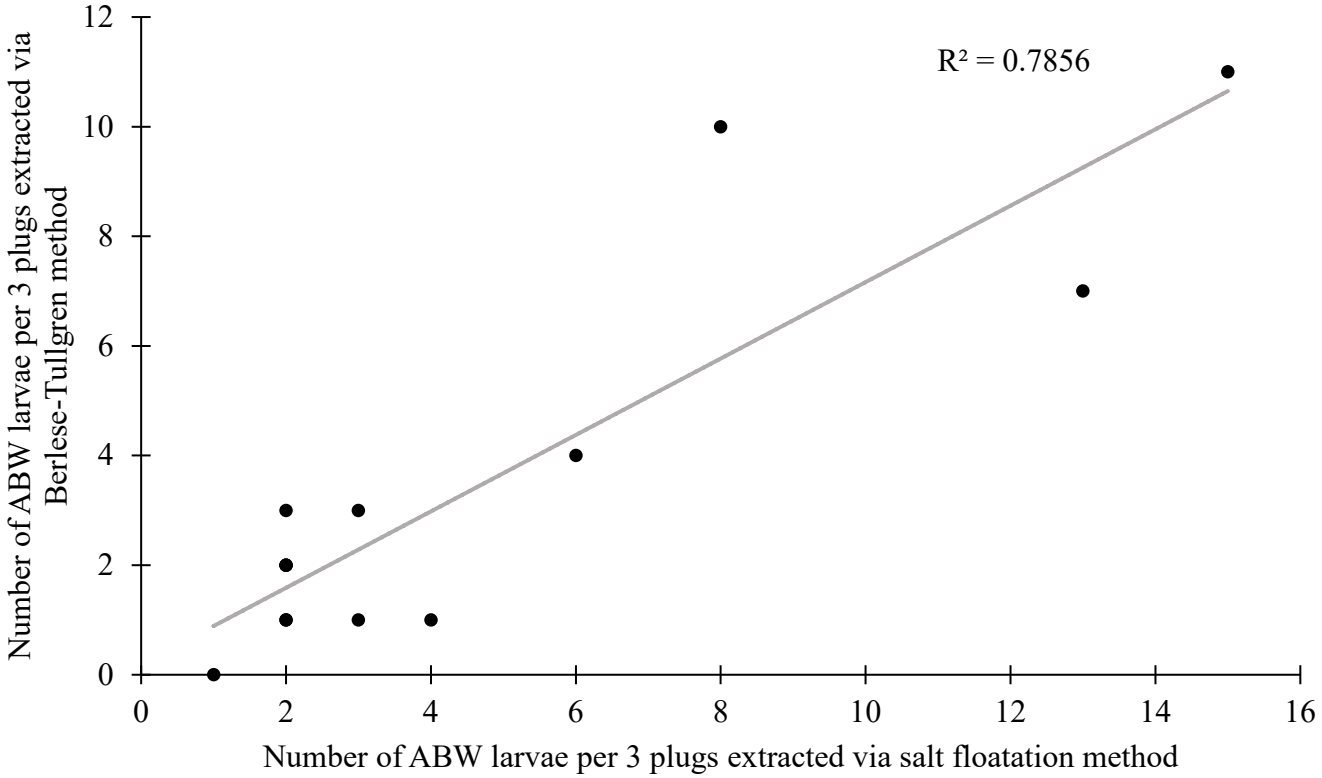

Figure 4.4. Linear relationship between annual bluegrass weevil (ABW) larvae extracted from turf cores using the standard salt float method versus a heat extraction using a Berlese-Tullgren funnel under an incandescent heat lamp. Each data point represents a sample of three turf cores taken from the same 1 meter^2 section of a golf course in southwest Virginia in summer 2020.

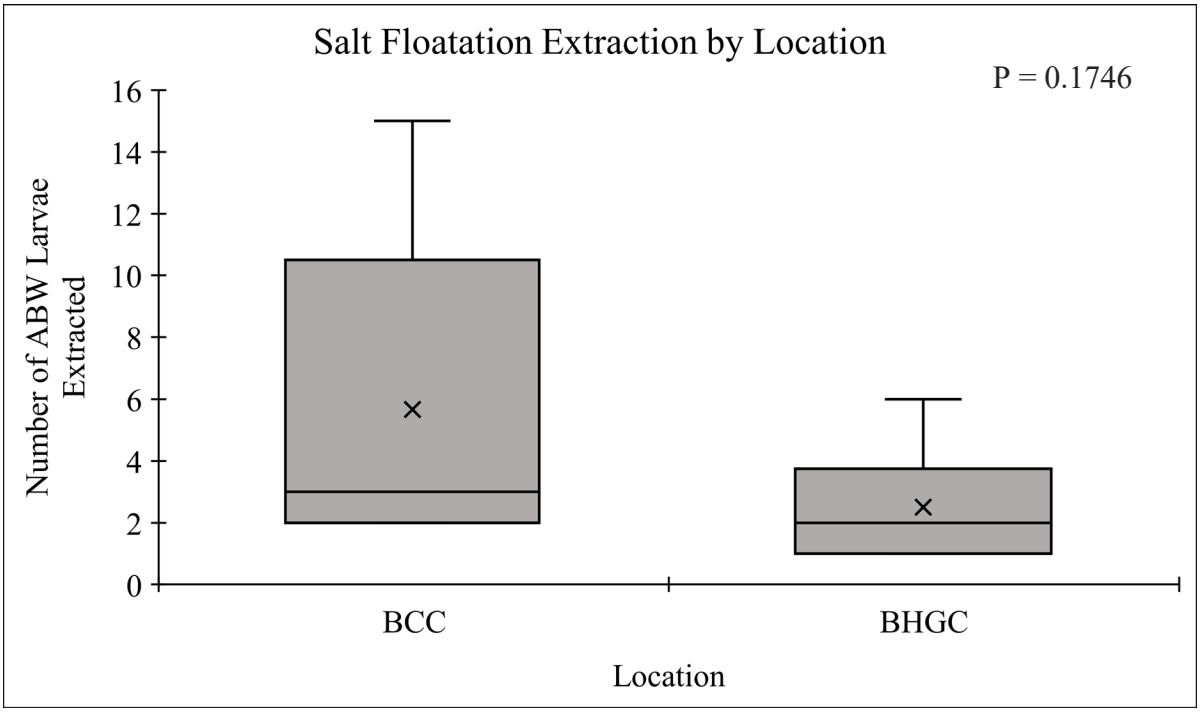

Figure 4.5. Number of annual bluegrass weevil (ABW) larvae extracted using the salt floatation method. Number of larvae extracted are separated by location: Blacksburg Country Club (BCC) or Ballyhack Golf Club (BHGC). The location had no significant effect on the number of larvae extracted via the salt floatation method.

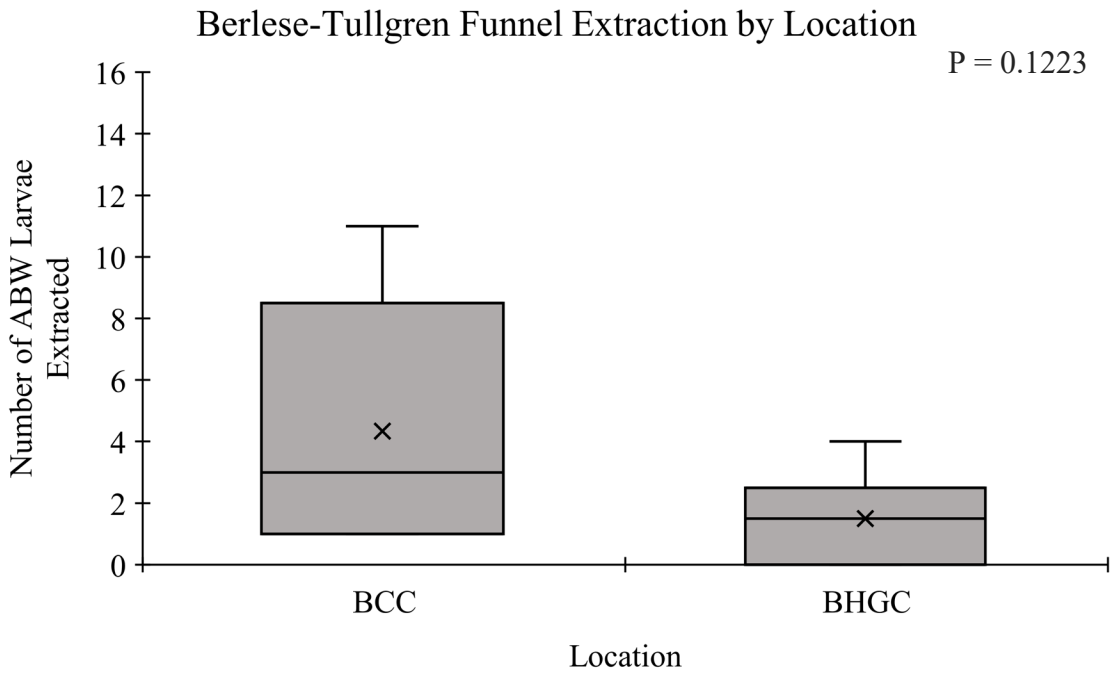

Figure 4.6. Number of annual bluegrass weevil (ABW) larvae extracted using the Berlese-Tullgren funnel method. Number of larvae extracted are separated by location: Blacksburg Country Club (BCC) and Ballyhack Golf Club (BHGC). The location did not have a significant effect on the number of larvae extracted via the Berlese-Tullgren funnel method.

Figure 4.7. Salt floatation larval extraction method product after one hour of settling time. Grass clippings, dirt, and debris make the identification of annual bluegrass weevil larvae, specifically earlier instar larvae, difficult.

Chapter 5. Susceptibility of Annual Bluegrass Weevils from Two Virginia Golf Courses to Bifenthrin and Commonly Applied Insecticides

Abstract

Annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is the most damaging insect pest of cool-season golf course turfgrasses in the northeastern and Mid-Atlantic US. Pyrethroid insecticides are commonly used by golf course superintendents to treat for ABW adults and resistance to this class of insecticide has become widespread in ABW populations in the northeastern US and cases are continuing to rise. No documented cases of pyrethroid resistance have been reported in Virginia ABW populations outside of the metropolitan Washington, D.C. area. In 2020, the susceptibility of ABW adults collected from two different Virginia golf course to the pyrethroid bifenthrin, along with other commonly used insecticides for ABW control and resistance management was evaluated. To determine the level of pyrethroid sensitivity, weevils from two different golf courses were subjected to varying rates of bifenthrin. Weevil populations from both golf courses exhibited complete mortality at 12.8 kg bifenthrin ha^{-1}, the field application rate 100-fold, suggesting complete resistance is not present. When testing other commonly used insecticides, trichlorfon, a larvicide, and chlorpyrifos resulted in significantly higher mortality rates than other insecticide treatments. Two other larvicides, spinosad and α-cypermethrin, were also found to have adult activity, an important factor to consider when treating for ABW and preventing pyrethroid, and other insecticide, resistance.

Introduction

Annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is the most damaging insect pest of cool-season golf course turfgrasses in the northeastern and Mid-Atlantic US (Peck et al. 2007). Annual bluegrass weevil primarily affects annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.), with *P. annua* being more susceptible to oviposition and damage than *A. stolonifera* (Kostromytska and Koppenhöfer 2014). The adult female ABW lays two to three eggs inside of the turfgrass plant sheath (Vittum 1999). Once the eggs hatch into larvae, the early instars burrow down inside the grass plant stem, feeding on the plant material. Once they become large enough, larvae drop out of the stem but continue to feed on the crown of the plant until they are ready to pupate in the thatch layer or soil (McDonald and Dernoeden 2007). The larval stage of ABW causes the majority of the damage exhibited, while the damage caused by adult feeding is considered negligible (Vittum 1999). Injury and damaged caused by ABW is generally expressed as small yellow-brown spots within the turfgrass canopy that eventually coalesce (Fig. 1.1). Once the spots have coalesced, damage can take on a "water-soaked" appearance (Vittum 1999). Because damage occurs during the summer, when *P. annua* and *A. stolonifera* are most susceptible to heat and drought, ABW damage is often confused for natural *P. annua* dieback or heat stress (Vittum 1999, McDonald and Dernoeden 2007).

Currently, chemical control is the only effective strategy for managing ABW (Vittum 1999, Vittum et al. 2012). However, there are several challenges to controlling ABW. The first is that adult ABW are treated using different insecticides than are used for larvae (Table 1). Second, ABW exhibits an asynchronous lifecycle; and thus, a variety of life stages of ABW can be present at any given time during the season (Figs. 3.1, 3.2). This complicates application

timing. While ABW larvae are responsible for causing the majority of the ABW damage seen on golf courses, golf course superintendents often apply insecticides for the adult stage to prevent oviposition, which are generally broad-spectrum insecticides such as pyrethroids, including bifenthrin or λ-cyhalothrin, or the organophosphate chlorpyrifos (McGraw and Koppenhöfer 2017). Because of this, ABW resistance to pyrethroids is becoming increasingly common in the northeast US and the Mid-Atlantic region alike, making treatment for ABW increasingly difficult in many locations (Ramoutar et al. 2009b, Ramoutar et al. 2009a, McGraw and Koppenhöfer 2017).

Instead of applying insecticides targeting the adults, larvicides such as trichlorfon (organophosphate), imidacloprid (neonicotinoid), spinosad (spinosyn), indoxacarb (oxadiazine), or cyantraniliprole (anthranilic diamide) can be used instead to reduce the possibility of pyrethroid resistance (Koppenhöfer et al. 2012). Although there are several active ingredients that can be applied for ABW larval control (Table 1), ABW resistance to pyrethroids is caused by enzymatic detoxification (Ramoutar et al. 2009a). Enzymatic detoxification is a nonspecific mechanism that breaks down the insecticide active ingredients before they can reach the target site (Williams 1947, Yu 2008). Because of the nonspecific mechanism of resistance, cross-resistance and decreased sensitivity to other non-pyrethroid classes of insecticides is possible from pyrethroid-resistant weevil populations (Koppenhöfer et al. 2018, Kostromytska et al. 2018). Although the number of cases of ABW resistance to pyrethroids is growing, some golf course superintendents apply pyrethroids as their sole means of ABW adult control as they have expressed that these insecticides cost much less than the other chemistries available on the market (McGraw and Koppenhöfer 2017).

Previous studies have demonstrated severe differences in ABW's susceptibility to pyrethroid insecticides, specifically in northeastern US populations (Cowles et al. 2008, Ramoutar et al. 2009b, Ramoutar et al. 2009a, Koppenhöfer et al. 2018, Kostromytska et al. 2018, Koppenhöfer et al. 2019). In a study conducted by Ramoutar et al. (2009a), specific pyrethroid LD_{50} values for northeastern weevils were determined. The LD_{50} ranges for bifenthrin and λ-cyhalothrin, two pyrethroids, were 1.80-244.67 ng insect^{-1} and 0.52-159.52 ng insect^{-1}, respectively. When ABW strains collected from southern New England golf courses were tested, they showed low to high levels of resistance to bifenthrin (6.1-125.9 fold) and λ-cyhalothrin (28.7-306.8 fold) (Ramoutar et al. 2009b). Selection by pyrethroids has left many courses with adult weevils that are unaffected by these chemistries (Cowles et al. 2008). In a study conducted by McGraw and Koppenhöfer (2017), the authors found that 20.1% of golf courses with damaging ABW populations (most distributed through New York, Connecticut, and New Jersey) reported having a pyrethroid-resistant ABW population either suspected or confirmed by a bioassay.

Once pyrethroid resistance has developed in an ABW population, continued use these chemistries will only exacerbate the resistance (Cowles 2010). In field studies (Cowles 2010), slight increases in mortality when combining pyrethroids with synergists, such as piperonyl butoxide, demethylation inhibiting fungicides, and gibberellin-blocking growth regulators (Ramoutar et al. 2010b), have not been statistically significant. Substituting pyrethroid use with selective larval insecticides can encourage predatory insects to keep ABW populations within manageable thresholds, instead of consequentially treating for them with pyrethroid applications (Cowles 2010).

The efficacy of pyrethroids for ABW control in Virginia is not known outside of the metropolitan Washington, D.C. area. The objectives of the pyrethroid and common insecticide studies were to determine the current susceptibility to bifenthrin, a commonly used pyrethroid, of ABW adults collected from Virginia golf courses, and to assess the susceptibility of other commonly used golf course turfgrass insecticides, respectively.

Methods and Materials

Weevil Collection

Adult ABW were collected from two golf courses in southwest Virginia, Blacksburg Country Club (Montgomery County) (37.213536, -80.359322) (BCC) in Blacksburg, VA, and Ballyhack Golf Club (Roanoke County) (37.227144, -79.890619) (BHGC) in Roanoke, VA. Weevils were extracted from the turfgrass via soap flushing as described by Koppenhöfer et al. (2020). At BCC, a fairway section (60% *P. annua*, 40% *A. stolonifera*) left untreated with insecticides was used for sampling for the pyrethroid study. For the common insecticide study, multiple areas of the golf course, both treated and untreated with insecticides, were used for weevil collection at BCC. At BHGC, a driving range target (5% *P. annua*, 95% *A. stolonifera*) left untreated with insecticides was used for collection in both studies. Lemon scented liquid dish soap (Ajax Ultra Super Degreaser Lemon Dish Liquid) was used as the soap flushing irritant at a rate of 7.8 mL soap L^{-1} water. The soapy water solution was applied to the turfgrass at a rate of 1 L mixture 0.09 m^{-2} (Koppenhöfer et al. 2020). The soapy water solution was randomly applied to sampling areas until enough weevils (>200) were collected. The weevils collected from soap flushing were placed in sealed plastic containers with grass clippings and transported unrefrigerated. All weevils were used the same day as collection and were only added to treatments if still living.

Sensitivity to Bifenthrin

Weevil adults collected from both BCC and BHGC were kept as separate populations. Each group was randomly assigned a treatment. Treatments included 0.128 kg bifenthrin ha^{-1}, 1.28 kg bifenthrin ha^{-1}, 12.8 kg bifenthrin ha^{-1}, to represent a 1x, 10x, and 100x field application rate, and were compared against a water control treatment (WCT). Each treatment was replicated four times with ten subsamples per replication. The treatments were administered via filter paper (Whatman Grade 43 90mm, Mfr. No. 28418057) that had been dipped in the insecticide solution (Farnsworth 1997). The filter paper was fully saturated with insecticide mixture and the excess insecticide mixture was allowed to drip from the paper. The filter paper was then placed inside of a glass Petri dish and the randomly assigned group of weevils were placed on top. The Petri dish lid was then placed on top to prevent weevils from escaping. The Petri dishes containing the saturated filter papers and weevils were placed in a randomized complete block design on a lab bench that did not receive direct sunlight and maintained at standard room temperature (21ºC - 26ºC) for 24 hours. After 24 hours, the mortality rates for each experimental unit were recorded.

Sensitivity to Common Insecticides

Adult weevils collected from BCC and BHGC were kept as separate populations. Weevils from each course were randomly separated into groups of ten. Each group was randomly assigned a treatment. Treatments included 0.128 kg bifenthrin ha^{-1} (Bifenthrin P, FMC Corp), 0.05 kg α-cypermethrin ha^{-1} (Fendona CS, BASF), 0.08 kg λ-cyhalothrin ha^{-1} (Scimitar, Syngenta), 9.21 kg trichlorfon ha^{-1} (Dylox 420 SL, Bayer), 0.05 kg chlorpyrifos ha^{-1} (Lorsban 4E, Corteva), 0.45 kg spinosad ha^{-1} (MatchPoint, Corteva), 0.45 kg imidacloprid ha^{-1} (Merit 75 WP, Bayer), and a water control treatment (WCT), with four replications per treatment. All rates used were based upon the label recommendations of the insecticides. If a range of rates were given for ABW treatment on the insecticide label, the higher end of the range was used. The

treatments were administered via filter paper (Whatman Grade 43 90mm, Mfr. No. 28418057) as previously described. Because mortality was measured on a percentage basis, additional weevils were added to the treatments following the groups of ten, bringing the total count of weevils per treatment to 10-15. The addition of extra weevils was dependent on the number of excess weevils collected from each golf course.

Data Collection and Analysis

Mortality rates were recorded 24 hours after initial insecticide exposure. The percent mortality for all insecticides, location, and their interaction were subjected to ANOVA. The bifenthrin study results were not statistically different by locations and were therefore pooled for further analyses. However, the common insecticide study results varied by location, and were compared by location. Thus, further analyses were run separate by location. Both data sets were subjected to ANOVA and means were separated using a Student's *t*-test ($\alpha = 0.05$) in JMP Pro 15 (SAS Institute, Cary, NC). The bifenthrin sensitivity data were graphed on a log scale and data were modeled using a non-linear regression 3-parameter (3P) logistic equation $\frac{c}{(1+Exp\left(-a\cdot(A.I.Concentration-b)\right))}$, where a = growth rate, b = inflection point, and c = asymptote (Fig 5.1) in JMP Pro 15. LC_{50} and LC_{90} were determined using a custom inverse prediction from this model.

Results

Sensitivity to Bifenthrin

Mortality rates of ABW adults subjected to bifenthrin were significant by concentration of active ingredient concentration ($p < 0.0001$). Mortality rates of ABW adults exposed to 0.128 kg bifenthrin ha^{-1}, the field application rate, ranged from 20%-80% at BCC and 50%-80% at BHGC. When exposed to 1.28 kg bifenthrin ha^{-1}, the 10x field application rate, mortality rates of

ABW adults at both locations were either 90% or 100%, with exposure to 12.8 kg bifenthrin ha^{-1}, or 100x the field application rate, exhibiting 100% mortality at both locations (data not shown). The effect of location ($p = 0.5580$) and the location by treatment interaction ($p = 0.7156$) were not significant. Therefore, data were pooled by location to model the effects of bifenthrin concentration. Data were modeled using a non-linear regression 3-parameter (3P) logistic equation $\frac{c}{(1+Exp\left(-a\cdot(A.I.Concentration-b)\right))}$ ($R^2 = 0.9254$) (Fig 5.1) due to the goodness of fit and the appropriateness of this equation for biological subjects. The lethal concentration of bifenthrin required for 50% mortality (LC_{50}) was 0.1071 kg ai ha^{-1}, according to the inverse prediction of our model. A concentration level of 0.1848 kg ai ha^{-1} was needed to kill 90% (LC_{90}) of adult ABW.

Sensitivity to Common Insecticides

Mortality rates of ABW adults subjected to common insecticides were significant by active ingredient, location, and location by treatment ($p < 0.0001$) (Table 5.1). Therefore, treatment data are presented by location. At BCC, all three pyrethroid treatments, bifenthrin, λ-cyhalothrin, and α-cypermethrin, performed similarly to each other (Fig. 5.2). Adult ABW exposure to the organophosphates, trichlorfon and chlorpyrifos resulted in the highest mortality. Imidacloprid resulted in low mortality similar to the WCT. Adult exposure to spinosad exhibited the largest range of mortality (10% - 83.33%) at the BCC location. Like BCC, trichlorfon and chlorpyrifos performed better than all other insecticides at BHGC (Fig. 5.3). However, at BHGC, α-cypermethrin had statistically lower mortality rates than the other two pyrethroids, bifenthrin and λ-cyhalothrin. Imidacloprid performed similarly to the WCT.

Discussion

Sensitivity to Bifenthrin

BCC and BHGC showed similar mortality curves and mortality rates from both locations were found to be statistically similar (P = 0.069). Weevil adults collected from both golf courses had 100% mortality after exposure to the 100x field application rate, 12.8 kg bifenthrin ha^{-1}. If survivability exists at the 100x field application rate, it is likely the weevils tested are exhibiting resistance to bifenthrin (Kostromytska and Koppenhöfer 2020). Because mortality was 100% at the 100x field rate, we conclude that these weevil populations do not have a high level of resistance. To reach 50% mortality rate, 0.1071 kg ai ha^{-1} is required, which is below the regular field application rate of 0.128 kg bifenthrin ha^{-1}. However, in order to achieve at least 90% mortality, a rate of 0.1848 kg ai ha^{-1} is needed, suggesting a decrease in sensitivity to bifenthrin at both locations. In order to mitigate this reduced sensitivity in the field, we recommend that larvicides be applied on a rotational cycle with only necessary adulticide applications, so as to reduce the overall possibility of resistance to other insecticides. Chlorpyrifos, an organophosphate, could also be used for adult treatment in lieu of pyrethroids, including bifenthrin, so long as it is used in a rotation with larvicides.

Sensitivity to Common Insecticides

Of the insecticides tested, α-cypermethrin, trichlorfon, spinosad, and imidacloprid are labelled for use on the larval stage of ABW, not the adult stage. While imidacloprid performed statistically similar to the WCT for controlling adult ABW, trichlorfon performed the same as the adult-labeled organophosphate chlorpyrifos at both locations. At BHGC, spinosad had a smaller range of mortality rates than BCC, from 60% to 80%. The data-based finding that labeled certain larval insecticides are exhibiting significant adult activity may prove important for golf course

superintendents. Because ABW is becoming increasingly difficult to control due to the rising cases of pyrethroid resistance, combined with an asynchronous life cycle, larvicides having adult activity will be important for golf course superintendents to remember when treating for ABW. While it was previously believed that application timing needs to be as precise as possible to target the appropriate life stage, an application of a larvicide, such as trichlorfon or spinosad, could very well help control the adult ABW population as well. It is also important to remember for resistance prevention. Because weevils can exhibit cross resistance within the pyrethroid class and between other insecticide classes, knowing which insecticides could be adding to this resistance is crucial for resistance prevention. Whereas previously, a larvicide was not thought to contribute to adult resistance, it is possible the larvicide influencing adults might foster resistance.

α-cypermethrin, a pyrethroid, is not labeled for ABW adults nor larvae. However, at both locations, α-cypermethrin treatments resulted in some adult activity. The α-cypermethrin treatment at BCC was statistically similar to the other two pyrethroids, both labelled for adult ABW. An insecticide, such as α-cypermethrin that is not labelled for ABW that is commonly used to treat other pests, such as sod webworm (*Crambus* sp.), black cutworm (*Agrotis ipsilon* Hufnagel), and billbug (*Sphenophorus* sp.) adults, will not often be thought of when trying to prevent resistance. Thus, knowing that some insecticides targeted for the larval stage and some not labelled for ABW at all do show a level of ABW control is pertinent for golf course superintendents.

In the large and growing effort to determine alternatives to pyrethroid insecticides, organophosphates should not be overlooked. Chlorpyrifos along with trichlorfon were more effective than all other insecticide treatments at both golf courses, suggesting that these older

chemistries might be beneficial in this control effort. However, chlorpyrifos specifically is being phased out of several states' agricultural industry. Hawaii and California have banned chlorpyrifos use and New York is currently phasing chlorpyrifos out of use (Solomon 2020). While chlorpyrifos is a viable alternative to pyrethroids for adult ABW control currently, the possibility of future bans should be considered when determining pyrethroid alternatives.

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Tables and Figures

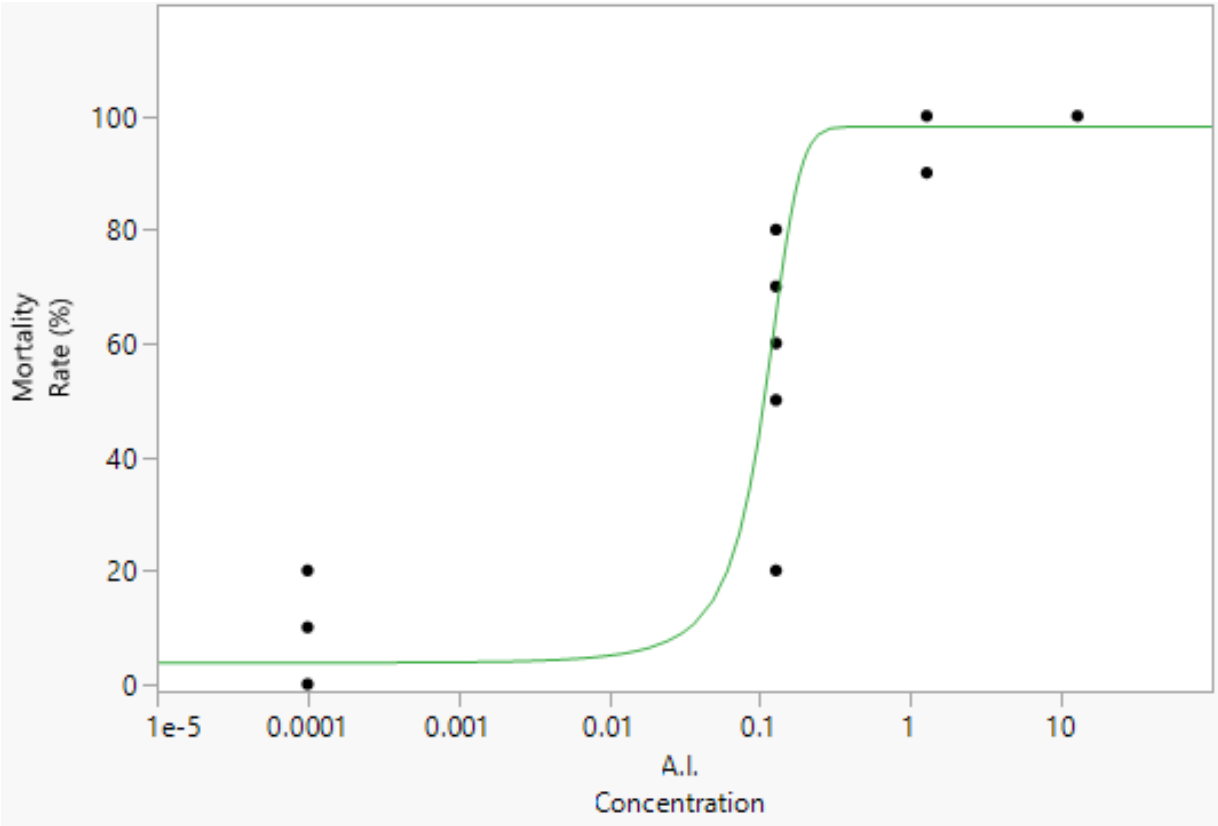

Figure 5.1. Bifenthrin mortality rate of annual bluegrass weevil adults as a function of active ingredient rate, graphed on a logarithmic scale with a 3-parameter (3P) logistic fit curve (R^2 = 0.9254). Weevils were collected from Blacksburg Country Club (Montgomery County VA) and Ballyhack Golf Club (Roanoke County VA) and mortality rates from both locations were pooled.

Table 5.1. Common insecticide Effects Test showing that treatment, location, and treatment by location all had significant effects on the mortality rates of the annual bluegrass weevil.

Source	DF	Sum of Squares	F Ratio	Prob > F
Treatment	7	80737.013	52.5380	<0.0001
Location	1	4646.714	21.1663	<0.0001
Treatment*Location	7	8163.838	5.3125	<0.0001

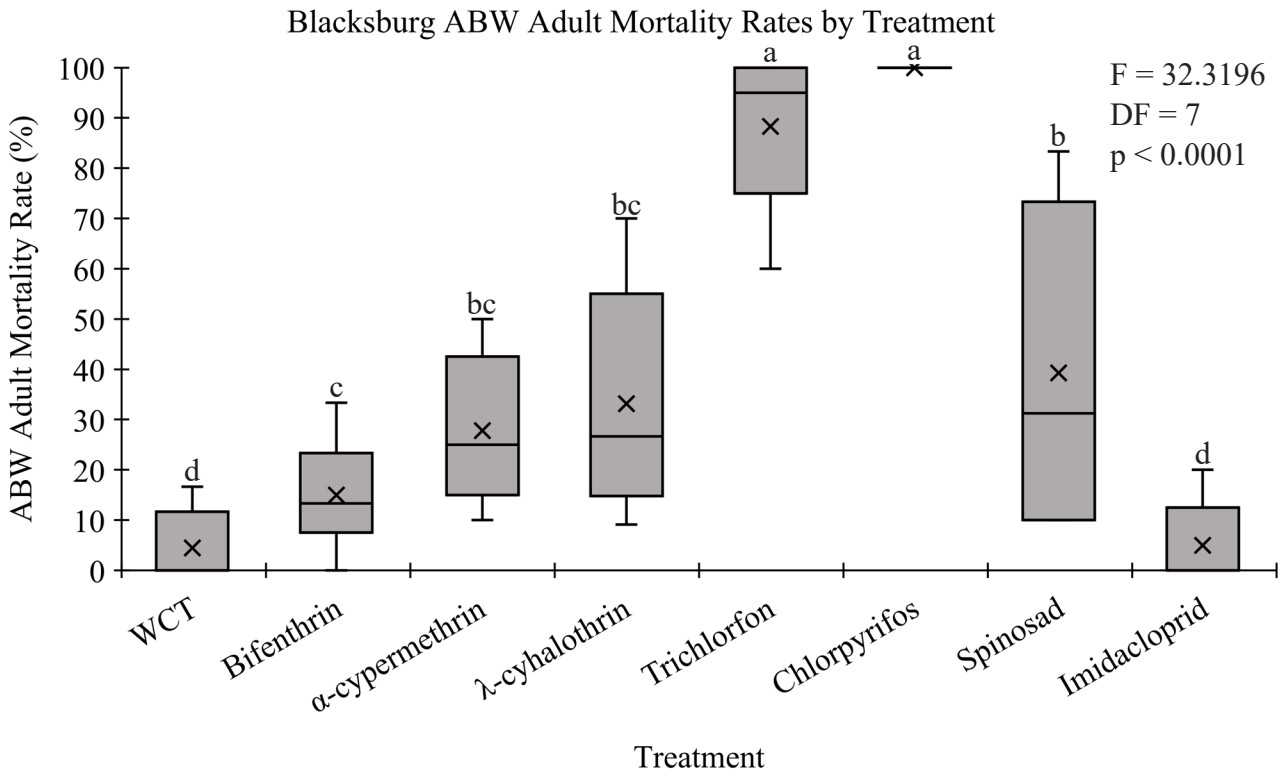

Figure 5.2. Common insecticide sensitivity study; Blacksburg Country Club annual bluegrass weevil (ABW) adult mortality rates as a function of treatment. Treatments not connected by the same letter are statistically different.

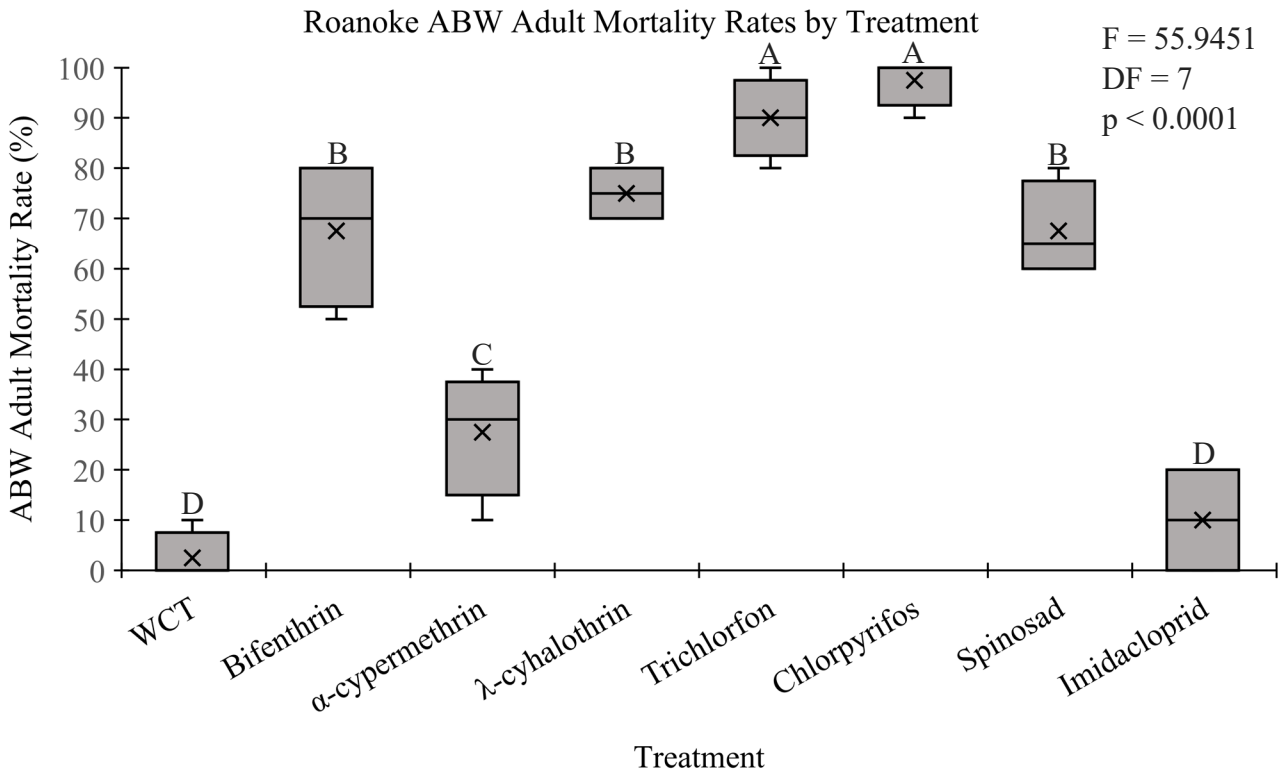

Figure 5.3. Common insecticide sensitivity study; Ballyhack Golf Club annual bluegrass weevil (ABW) adult mortality rates as a function of treatment. Treatments not connected by the same letter are statistically different.

Thesis Conclusion

Annual bluegrass weevil (ABW), *Listronotus maculicollis*, has been one of the most challenging insect pests of golf course turfgrass in the northeastern United States for decades. Although confined to the northeastern states for many years, this insect species is spreading and, based on my survey of golf course superintendents and visits to selected Virginia golf courses (Chapter 2), as well as previous published records, ABW is now widely established on a majority of golf courses in Virginia with primarily cool season turfgrasses, particularly annual bluegrass (*Poa annua* L.) and/or creeping bentgrass (*Agrostis stolonifera* L.).

Adult ABW emerge from overwintering in the early spring. In the northeast, the peak of spring adult activity typically occurs in April. Based on two years of sampling three golf courses (third course data not included) in Virginia in 2019 and 2020, I found that there was high adult activity occurring in March. Moreover, dissected female ABW collected in late March had mature ovaries typical of the ovipositional period. This information is important for golf course managers as it indicates crucial timing for early adulticide applications in VA. Removing any egg-laying adult females from the population will help reduce future larval infestations feeding on the grass plant crowns and roots. The first larval peaks of ABW typically occurred in late April and this is the suggested time of initial larvicide applications in VA. I also showed the likely presence of three generations of ABW in VA. It is likely that adult and larval activity continues into September and possibly October based on the data retrieved on the last day of sampling each year, and thus a fourth generation may be occurring.

Current recommendations for extraction and sampling of ABW larvae involve collecting soil plugs, breaking them apart in a saltwater solution, and counting the number of larvae that float to the surface. Detecting early instar larvae and sifting through numerous tiny grass clippings on the water surface are drawbacks of this method. In 2020, I compared the salt floatation technique with the use of heat extraction via a Berlese-Tullgren funnel which dries the soil plugs and forces ABW larvae downward through the soil and into a catch jar in order to escape the extreme heat. I used soil plugs collected from two different golf courses in Virginia with established populations of ABW. Larvae from matched pairs of plant and soil cores were extracted using either the salt floatation method or the Berlese-Tullgren funnel method. Results showed that the two extraction methods were highly correlated with regards to the number of ABW larvae collected, and there was no significant difference between the methods in the number of larvae extracted. This research suggests that both methods are comparable for extracting ABW larvae.

Effective control of ABW using insecticides can be challenging because of insecticide resistance problems, particularly to pyrethroids (IRAC Group 3). Although cross-resistance to other insecticides has also occurred in ABW populations from the northeastern U.S., because we do not know where our ABW populations on Virginia golf courses originated from, it is possible that pyrethroid resistance genes may be present in our VA ABW populations already. In 2019, I collected several populations of ABW adults from Virginia golf courses and tested them for pyrethroid susceptibility using a filter paper dip bioassay. With this bioassay, a typical spray tank concentration of the pyrethroid bifenthrin was applied to filter paper discs and compared with a water dipped control filter paper placed in Petri dishes. Weevil adults were placed in the dishes

and were maintained on the treated filter paper. This assay should result in 100% mortality of weevils in 24 hours as pyrethroids have rapid activity on insects leading to a quick death. Based on ABW populations collected from Harrisonburg, Stanardsville, Nokesville, Crozet, Roanoke, and Blacksburg, mortality ranged from 32.5% to 100% with a lot of variation. This is an indication that our Virginia ABW populations may likely have different levels of pyrethroid resistance.

In 2020, I evaluated the susceptibility of ABW adults collected from two different VA golf courses to the pyrethroid bifenthrin, along with other commonly used insecticides. To determine the level of pyrethroid sensitivity, weevils were subjected to varying rates of bifenthrin. Weevil populations from both golf courses exhibited complete mortality at 12.8 kg ai ha^{-1}, the field application rate 100-fold, suggesting complete resistance is not present. When testing other commonly used insecticides, trichlorfon, a larvicide, and chlorpyrifos resulted in significantly higher mortality rates than the other insecticide treatments. Two other larvicides, spinosad and α-cypermethrin, were also found to have adult activity. This is an important factor to consider when treating for ABW and preventing pyrethroid, and other insecticide, resistance.

After two and a half years of researching ABW populations in VA, I feel that we now have a better understanding of the distribution, seasonal biology, and management of this pest. Such information will be important for golf course superintendents in Virginia and other more southern states where this pest species is spreading.

Appendix A

Figure A1. Blacksburg Country Club adult and larval annual bluegrass weevil data, with larvae separated into small larvae, larval instars 1-3, and large larvae, larval instars 4-5, from the 2019 season. The use of "N.D." denotes no data were recorded.

Date (m/dd/yyyy)	Cumulative GDD	Adults 0.092 m^{-2}	Small Larvae 0.092 m^{-2}	Large Larvae 0.092 m^{-2}
2/28/2019	0	0	N.D.	N.D.
3/13/2019	1.5 (+1.5)	0	N.D.	N.D.
3/19/2019	11 (+9.5)	10.33	N.D.	N.D.
3/28/2019	11 (+0)	9.66	N.D.	N.D.
4/3/2019	25 (+14)	17.33	N.D.	N.D.
4/10/2019	83 (+58)	14	0	0
4/17/2019	137.5 (+54.5)	8	0	0
4/23/2019	185 (+47.5)	5	0	0
4/29/2019	253 (+68)	2.66	0	0
5/8/2019	400 (+147)	3.33	27.2	0
5/15/2019	475 (+75)	6	40.8	0
5/23/2019	615 (+140)	1.33	0	95.2
5/31/2019	801 (+186)	5.66	0	27.2
6/7/2019	917.5 (+116.5)	3.66	0	0
6/14/2019	1017 (+99.5)	33.33	0	0
6/21/2019	1159.5 (+142.5)	11	0	40.8
6/28/2019	1306.5 (+147)	25.7	40.8	81.6
7/7/2019	1540 (+233.5)	18.3	0	54.4
7/23/2019	1954.5 (+414.5)	48	27.2	136
8/1/2019	2144.5 (+190)	24.7	13.6	13.6
8/5/2019	2236.5 (+92)	8.5	N.D.	N.D.
8/13/2019	2424.5 (+188)	14	40.8	40.8
8/29/2019	2789 (+364.5)	9	54.4	0

Figure A2. Blacksburg Country Club adult and larval data, with larvae separated into small larvae, larval instars 1-3, and large larvae, larval instars 4-5, from the 2020 season. The use of "N.D." denotes no data were recorded.

Date (m/dd/yyyy)	Cumulative GDD	Adults 0.092 m^{-2}	Small Larvae 0.092 m^{-2}	Large Larvae 0.092 m^{-2}
3/3/2020	1.5	1.3	N.D.	N.D.
3/10/2020	10 (+8.5)	4.3	N.D.	N.D.
3/19/2020	39 (+29)	4	N.D.	N.D.
3/25/2020	52.5 (+13.5)	4	N.D.	N.D.
4/2/2020	113.5 (+61)	7.3	N.D.	N.D.
4/9/2020	163 (+49.5)	12.66	N.D.	N.D.
4/14/2020	175.5 (+12.5)	12.5	N.D.	N.D.
4/23/2020	184.5 (+9)	16	27.2	0
5/1/2020	211.5 (+27)	13.8	136	0
5/7/2020	252 (+40.5)	6.7	68	0
5/15/2020	281.5 (+29.5)	6.7	27.2	13.6
5/29/2020	483.5 (+202)	2.3	95.2	122.4
6/4/2020	595 (+111.5)	2.3	0	0
6/11/2020	749 (+154)	10.7	27.2	68
6/23/2020	941 (+192)	8.7	0	27.2
7/1/2020	1111 (+170)	6.7	13.6	0
7/7/2020	1265.5 (+154.5)	1.7	108.8	95.2
7/13/2020	1417 (+151.5)	7	27.2	27.2
7/22/2020	1668.5 (+251.5)	8.33	40.8	27.2
7/29/2020	1859 (+190.5)	9.33	27.2	27.2
8/4/2020	2007.5 (+148.5)	17.67	81.6	13.6
8/11/2020	2179 (+171.5)	21.6	13.6	13.6
8/18/2020	2327 (+148)	53.67	54.4	13.6
8/26/2020	2508.5 (+181.5)	29	13.6	13.6
9/3/2020	2703 (+194.5)	13.33	13.6	13.6
9/10/2020	2837.5 (+134.5)	8.67	13.6	0
9/17/2020	2948.5 (+111)	7.33	0	13.6

Figure A3. Ballyhack Golf Club adult and larval data, with larvae separated into small larvae, larval instars 1-3, and large larvae, larval instars 4-5, from the 2019 season. The use of "N.D." denotes no data were recorded.

Date (m/dd/yyyy)	Cumulative GDD	Adults 0.092 m^{-2}	Small Larvae 0.092 m^{-2}	Large Larvae 0.092 m^{-2}
2/28/2019	0	2	N.D.	N.D.
3/13/2019	8.5 (+8.5)	9.33	N.D.	N.D.
3/19/2019	27.5 (+19)	8	N.D.	N.D.
3/28/2019	35.5 (+8)	9.33	N.D.	N.D.
4/2/2019	58.5 (+23)	8	N.D.	N.D.
4/10/2019	141 (+82.5)	10.6	0	0
4/17/2019	221.5 (+80.5)	7	0	0
4/23/2019	292 (+70.5)	22.66	0	0
4/29/2019	378 (+86)	8.33	0	0
5/8/2019	560.5 (+182.5)	1.33	95.2	0
5/15/2019	669.5 (+109)	5	0	0
5/23/2019	849.5 (+180)	6	0	27.2
5/31/2019	1077.5 (+228)	20.33	0	0
6/7/2019	1228 (+150.5)	40.66	0	0
6/14/2019	1354 (+126)	11.66	27.2	13.6
6/21/2019	1532.5 (+178.5)	3	0	27.2
6/27/2019	1686.5 (+154)	28	13.6	68
7/7/2019	1989.5 (+303)	8	0	81.6
7/23/2019	2468 (+478.5)	5	13.6	54.4
7/31/2019	2675.5 (+207.5)	12.3	0	40.8
8/5/2019	2808 (+132.5)	5.3	N.D.	N.D.
8/13/2019	3022 (+214)	12.33	54.4	108.8
8/29/2019	3448 (+426)	6	0	136

Figure A4. Ballyhack Golf Club adult and larval data, with larvae separated into small larvae, larval instars 1-3, and large larvae, larval instars 4-5, from the 2020 season. The use of "N.D." denotes no data were recorded.

Date (m/dd/yyyy)	Cumulative GDD	Adults 0.092 m^{-2}	Small Larvae 0.092 m^{-2}	Large Larvae 0.092 m^{-2}
3/3/2020	5.5	2.3	N.D.	N.D.
3/10/2020	29.5 (+24)	22	N.D.	N.D.
3/19/2020	82 (+52.5)	12	N.D.	N.D.
3/25/2020	113 (+31)	26.3	0	0
4/2/2020	196 (+83)	7.3	0	0
4/9/2020	281.5 (+85.5)	11.33	0	0
4/18/2020	307.5 (+26)	4.5	0	0
4/23/2020	331.5 (+24)	6	0	0
5/1/2020	390.5 (+59)	11	54.4	0
5/7/2020	450 (+59.5)	7.3	68	13.6
5/15/2020	496 (+46)	7.3	27.2	40.8
5/29/2020	735 (+239)	7.7	0	0
6/4/2020	879 (+144)	2.33	0	0
6/11/2020	1070 (+191)	11.7	0	13.6
6/23/2020	1302 (+232)	21.33	13.6	0
7/7/2020	1704.5 (+402.5)	3.33	40.8	54.4
7/13/2020	1888.5 (+184)	3	13.6	27.2
7/22/2020	2185 (+296.5)	1	10.2	51
7/29/2020	2409.5 (+224.5)	9	27.2	0
8/4/2020	2587 (+177.5)	23.67	0	13.6
8/11/2020	2781 (+194)	20	40.8	13.6
8/18/2020	2953 (+172)	43.33	27.2	27.2
8/26/2020	3171.5 (+218.5)	28	68	81.6
9/3/2020	3400 (+228.5)	12.33	13.6	27.2
9/10/2020	3567 (+167)	16	0	13.6

Appendix B

Table B1. Extraction results separated by method with location distinguished in the leftmost column. Each row represents two sampling groups, one sampling group subjected to the salt floatation method and one sampling group subjected to the Berlese-Tullgren funnel method, that were taken at the same time from the same m^2 of area. Each sampling group consists of three turfgrass plugs added together for the sampling group total.

	Number of Larvae Extracted by Method	
Location	Salt Floatation Method	Berlese-Tullgren Funnel Method
BCC	2	3
BCC	2	1
BCC	15	11
BCC	13	7
BCC	8	10
BCC	2	1
BCC	3	3
BCC	4	1
BCC	2	2
BHGC	1	0
BHGC	2	2
BHGC	6	4
BHGC	2	2
BHGC	3	1
BHGC	1	0