

Developing a remote sensing approach for integrated pest and pollinator management in  
turfgrass

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# **Developing a remote sensing approach for integrated pest and pollinator management in turfgrass**

**By Shannon Bradley**

**Abstract: (Academic)**

Golf courses can expand hundreds of acres, making scouting for both pests and beneficial insect populations a time-consuming task. Scouting for insects is labor-intensive, potentially damaging, but is an integral part of an integrated pest and pollinator management (IPPM) plan. Virginia golf courses are currently using remote sensing and light reflectance to detect non-insect pests in turfgrass. This thesis aims to develop remote sensing and light reflectance methods to aid in a turfgrass IPPM plan, to document the phenology of ABW weevil (*Listronotus maculicollis* Kirby, Coleoptera: Curculionidae, ABW), and to catalogue pollinator-friendly out-of-play areas. Light reflectance, the measurement of the amount of light reflected, of plants can be used as a proxy for the health of a plant. The light reflectance of turfgrass affected by ABW stress and plants in the out-of-play areas of golf courses was collected proximally and remotely, using a backpack spectrometer and an unmanned aerial vehicle (UAV), respectively. Mathematical light reflectance indices were applied and compared to insect populations in both areas to determine the correlation. The Normalized Difference Vegetation Index (NDVI), which uses red and near-infrared wavelengths to indicate stress, was found to highlight ABW stressed turfgrass. The Structure Intensive Vegetation Pigment Index (SIPI), which uses red and green wavelengths to highlight flowering plants, was found to highlight potential pollinator-friendly habitats in out-of-play areas. When applied to flights, NDVI could help in the targeted application of insecticides to combat the annual bluegrass weevil, therefore reducing their presence in the environment. The use of SIPI could highlight potential pollinator friendly habitats and therefore assist superintendents in the development of their IPPM plan.

# **Developing remote sensing approaches for integrated pest and pollinator management in turfgrass**

**By Shannon Grace Bradley**

## **Abstract: (General Audience)**

Scouting, such as completing visual monitoring or taking soil core samples, is an important part in the development of an integrated pest and pollinator management (IPPM) plan for Virginia golf courses; an IPPM plan focuses on control of a pest, while considering the needs of pollinators. The size of golf courses makes scouting for insect pests and beneficial insects a time-consuming task. Golf courses are currently using remote sensing, the use of drones in combination with other technology, to scout for other pests or disease. Light reflectance, the measurement of the amount of light reflected, is often used in combination with remote sensing as a proxy for the health of plants. This thesis developed remote sensing and light reflectance techniques not only to detect a common turfgrass pest, the annual bluegrass weevil (*Listronotus maculicollis* Kirby, Coleoptera: Curculionidae, ABW), but to also predict the presence of potential pollinator habitats in the out-of-play areas of Virginia golf courses. Instruments such as a spectrometer and a drone were used to collect light reflectance at the ground level and aerially, respectively. Ground data was collected through soap water flushes to detect adult ABW, and visual monitoring of potentially pollinating bees, beetles, butterflies, and flies. The light reflectance and ground data were compared using mathematical indices to determine if there was a relationship between the presence of insects and a particular index. Indices could be applied to drone flights that golf course superintendents are already performing, and they can use this information to highlight potential areas of insect presence. This will help them to take care not to apply insecticides in areas with pollinators or to only apply necessary insecticides where there is likely a presence of ABW. This will reduce the labor, other costs, and the environmental impact of insecticides.

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## **Chapter 1: Introduction of insects in an integrated pest and pollinator management plan**

The research presented in this thesis focused on the phenology, monitoring, and detection of the annual bluegrass weevil (*Listronotus maculicollis* Kirby, Coleoptera: Curculionidae, ABW), as a key pest of turfgrass in golf courses. Additionally, it focused on the use of remote sensing as a method of cataloging and monitoring out-of-play areas with the potential to serve as pollinator habitats. Remote sensing and the measurement of light reflectance are currently used together as a method of detection and monitoring of turfgrass diseases and pests. There is a need for monitoring and detection methods that do not damage turfgrass, as current ABW larval scouting methods are destructive and time consuming. Likewise, there is a need for a monitoring technique to predict potential pollinator friendly habitats in the out-of-play areas of golf courses, as these areas are not always physically accessible for scouting.

The ABW is a pest of annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.), common cool-season turfgrass species that are used in manicured and highly maintained settings. This insect was first reported as a pest of turfgrass in 1931 in Connecticut and has been a problem in New York since 1950's (Vittum 1999). Even though it is reported in more than 30 states, it continues to be an issue in the northeast and mid-Atlantic states, including NC and VA (Vittum 1999, Daly 2021). Due to an increased presence of ABW, there is a need to understand the phenology of this pest at Virginia golf courses. Currently, the method used for ABW larvae scouting, soil core sampling, is a destructive yet crucial method for adult emergence prediction in northern states (Daly 2021). There is a need for the development of a method that does not damage the turfgrass, including Virginia, where larvae are not as accurate as a predictor. Understanding when this pest emerges and how it disperses could help decrease the usage of insecticides, which are currently the most commonly used method of combatting ABW (McGraw 2017). Insecticides such as pyrethroids are used to manage this pest, but pyrethroid resistance has been found in populations of ABW (Ramoutar et al. 2009, McGraw et al. 2017), being a potential issue in Virginia.

Golf courses are ecosystems that contain both pests and beneficial insects, such as pollinators. Superintendents, golf professionals, and other stakeholders have become more conscious of pollinating insects following the 'Making Room for Native Pollinators' and 'How to Create Habitat for Pollinator Insects on Golf Courses' initiatives. These initiatives were released in part by the Xerces Society and detail the establishment of native pollinator-friendly spaces in the landscape (Shephard et al. 2002). Out-of-play areas at golf courses have the potential to serve as pollinator habitats. These spaces are often full of flowering plants, due to their lack of disturbance and low inputs; but are not necessarily accessible to traverse by the golf course personnel. Additionally, out-of-play areas may cover large portions of a golf course (Lyman et al. 2007), therefore there is a need for a method of highlighting existing areas with potential to serve as pollinator habitats. Remote sensing, specifically unmanned aerial vehicles (UAV), could offer a method that covers larger areas and is not limited to where personnel can safely move. The use of remote sensing and light reflectance to highlight areas of plants that could attract pollinators would help golf course superintendents in developing their integrated pest and pollinator management (IPPM) plan. Overall, access to remote sensing and light reflectance information could help reduce or target specific areas in which insecticides might be needed to control high populations of a pest. This would not only decrease the cost of

insecticides and their amounts in the environment, but also help in ensuring that potential pollinator areas are protected from non-target insecticide application effects.

Several projects for this research were conducted at golf courses in central and eastern Virginia. There was also an outreach nature for this research, where sharing information with the stakeholders and public regarding ABW and remote sensing was deemed appropriate. The first chapter of this thesis is comprised of two Virginia Cooperative Extension (VCE) publications that describe the insects of interest and the methods that were used to monitor them (see those two VCE publications below). The first publication is titled “The Annual Bluegrass Weevil as a Golf Course Pest in Virginia” and includes scouting techniques commonly used for monitoring of this pest as well as the phenology. The second extension publication title is “Pollinators in the Out-of-Play Areas of Virginia Golf Courses” and features information about monitoring pollinator habitats with light reflectance. These publications, which are written using accessible language, have been included to act as an introduction for topics addressed in the second and third chapters of this thesis.

The second and third chapters of this thesis expanded on the topics presented in the “The Annual Bluegrass Weevil as a Golf Course Pest in Virginia” section of the first chapter. The second chapter covered the phenology of the annual bluegrass weevil, as determined through soap water flushes (Koppenhofer et al. 2020) and soil core samples (Daly 2021) at three golf courses in Virginia for two years. In addition to the phenology of this pest, the third chapter looks at the light reflectance of turfgrass infested with ABW in a controlled greenhouse environment. This light reflectance was then compared to light reflectance collected both proximally and via UAV (unmanned aerial vehicle) under golf course settings. This data was all compared and correlated to the Normalized Difference Vegetation Index (NDVI), a light reflectance index that uses red and near infrared to detect areas of stress (Aparicio et al. 2000), which is already employed by the green industry.

The project presented in the fourth chapter collected information past the fairway and focused on the out-of-play areas as potential pollinator friendly habitats. The potential services of these areas had not been previously catalogued in Virginia. This fourth chapter described the types of insects and flowering plants present in those areas. Additionally, the light reflectance of sampling points in out-of-play areas was collected both proximally (with a hand-held radiometer) and remotely (using an UAV). The collected light reflectance was used to test the relationship between six different mathematical reflectance indices and the presence of pollinators. The ultimate goal was to propose an index suitable to highlight areas attracting high populations of pollinators under golf course conditions.

## Introduction

The annual bluegrass weevil (ABW; *Listronotus maculicollis*, Coleoptera: Curculionidae) is an expensive pest of cool season grasses, such as poa (*Poa annua*) and creeping bentgrass (*Agrostis stolonifera*). In recent years, ABW have become a more prominent pest of golf courses in Virginia.

## Description

ABW are small beetles measuring about 3-5 mm (3/32 – 3/16 inch) with an extended snout that is characteristic of these adults (Fig. 1). Female ABW are slightly larger than male ABW, though this difference in size is anecdotal. Adult ABW can range in color from a reddish brown to a black color, as they darken through a process called sclerotization that occurs as their exoskeleton hardens. ABW larvae are white or cream in color and have a brownish head capsule with no legs (Fig. 2). They range from about 1-3 mm (1/25 – 3/16 inch) depending on their life stage.

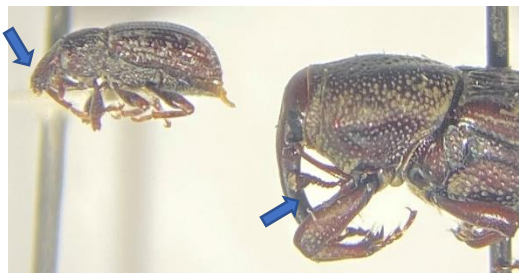


Figure 1. An ABW is pictured on the left with the antennae higher on the snout towards the head. A billbug weevil is pictured on the right with antennae that sit lower on the snout, further from the head. Photo by Shannon Bradley.



Figure 2. Left to right: ABW adult and immatures. Beneath the ABW is the tip of a pen for reference of their small size. Photo by Shannon Bradley.

## Life Cycle

ABW can be found from February to November in Virginia, which differs from more northern states, as the warm weather offers a longer season for this pest. ABW overwinter as adults in leaf litter near trees and then emerge and move towards the rough of the golf course as weather warms. While numbers can be found throughout this time frame, the largest densities of ABW populations are expected between the end of May and the beginning of July (Fig. 3). These peaks in the population of adults are then often followed by damage caused by immatures. Immature ABW have five instars, each taking 5-7 days to develop.

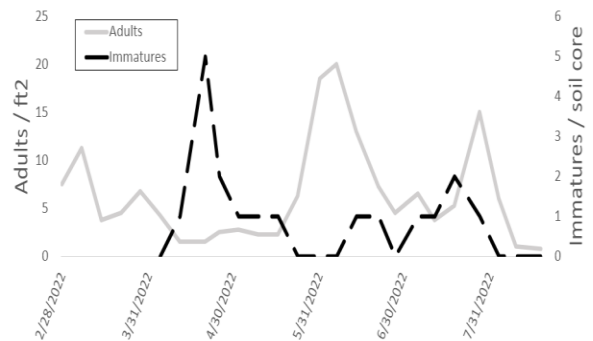


Figure 3. Adult and immature populations collected from a golf course in Richmond, VA in 2022. Immature

populations peaked in late-April and adult populations peaked about six weeks later in early June.

### Damage

Most turfgrass damage caused by ABW is the result of immature feeding, though the adults could also feed on the turf blades. Immature ABW feed along the sheath of the leaves and eventually shift their feeding to the roots of the turfgrass. These feeding habits cause an uneven surface, and the infested turfgrass often yellows or browns. As with many stressors of turfgrass, ABW damage often starts as a small area of brown and then continues to spread. This damage can appear to be stress caused by lack of water (Fig. 4). Due to the high standard of care for turfgrass under golf course settings, this browning and unevenness is unacceptable.



Figure 4. Left side shows turfgrass with large populations of ABW found in July 2022. The right side shows the same spot six weeks later. Photos by Shannon Bradley

### Impacts of Damage

Monitoring and management of this pest is essential to avoid large areas of highly damaged turfgrass. The ABW is a major pest, not only in terms of the damage it causes, but also in the management plans which often present a large expense. Golf course superintendents in Virginia have reported spending up to \$7,000 annually to

treat ABW. Consistent monitoring of the greens is critical to early detect infestations of this pest.

### Monitoring

Adult ABW are monitored using a soap water flush, in which lemon-scented dish soap is an irritant, driving adults from the turfgrass (Fig. 5 and Table 1). This method is affordable, does not damage the turfgrass, and is an effective way of monitoring the presence of adults.

To complete a soap water flush, combine soap and water following the ratio provided in Table 1. Pour the mixture over one squared foot (0.31 m<sup>2</sup>) area and let sit. After one minute, observe the area for the presence of adult ABW, which will often climb to the top of turfgrass blades. Repeat pouring, waiting, and observing (suggested to do two flushes on the same selected spot). Additional adults may be collected after this second flush which will saturate the soil. Plan to do flushes over at least three different sections of the green.

Immature ABW are monitored using soil cores (7.62 cm of diameter by 8.89 cm in length, 3 by 3.5 inches) taken from suspect areas. These soil cores are then placed under heat lamps for 24 hours. The heat from the lamps causes the immature ABW to move away from the heat and fall into a container with ethanol. When heat lamps are unavailable, immature ABW can be scouted using saltwater floats. Soil cores are also taken for this method and are broken apart and shaken in a saltwater mixture. Due to the density of the immature ABW, they are expected to float to the surface of the mix.

Table 1. Proportions of salt or soap to water for ABW monitoring.

Scouting Method	Life Stage	Time (min)	m L or g	Water (Liter)
Soap Flush	Adult	2	30	3.79
Saltwater Float	Immature	15	170	1

Note: Time is in reference to the waiting period needed before proceeding to collect ABW counts.

Both methods that monitor for immature ABW require that turfgrass is removed and inspected because larvae are often found in the soil and in lower portions of the turfgrass. As an alternative tool, light reflectance could be a nondestructive method of monitoring for both adult and immature ABW under golf course conditions.



Figure 5. A handful of ABW collected from a soap water flush. Photo by Shannon Bradley.

### Light Reflectance for Monitoring ABW

Light reflectance is the measurement of the quantity of light that is reflected off from an object and is collected with an instrument called a spectrometer (Fig. 6).

Light reflectance signatures (Fig. 7) can be used to determine the overall health of a plant, with healthier plants having higher reflectances.

Mathematical indices can also be calculated using the reflectance gathered at different wavelengths to highlight specific characteristics of the plant such as chlorophyll content or foliar damage.

Normalized Difference Vegetation Index, NDVI, is an index commonly used to highlight stress in turfgrass using red and near-infrared wavelengths. A preliminary analysis has shown a negative relationship between NDVI and ABW densities. The ultimate goal is for golf course superintendents to use light reflectance and identify 'hot spots' of ABW populations.



Figure 6. The spectrometer in use (backpack) at a golf course, collecting light reflectance. Photo by Joseph Leo.

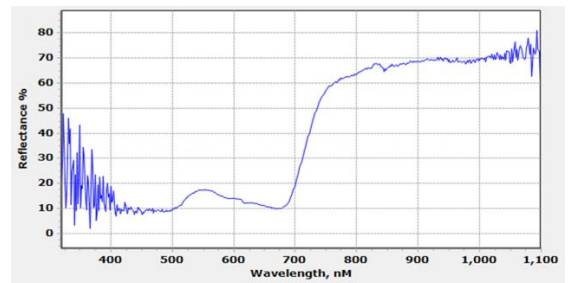


Figure 7. An example of light reflectance signature taken from turfgrass under field conditions.

## Introduction

Pollinator preservation efforts have increased in recent years through the establishment of pollinator-friendly habitats (Fig. 1). Virginia golf courses offer potential pollinator friendly spaces in the out-of-play areas. These out-of-play areas are the spaces beyond the maintained



Figure 1. Monarch butterfly (*Danaus plexippus*, Lepidoptera) photographed in a native out-of-play area in Williamsburg, VA. Photo by Shannon Bradley.

turf of a course, which can provide the food and shelter to pollinators that turfgrass could lack. There is limited information about the plant and insect makeup of these potential pollinator habitats in a golf course. Remote sensing, including the use of drones and light reflectance, offers the potential to both identify and monitor the presence and health of these potential pollinator habitats.

## Pollinators

Pollinators are animals that support an ecosystem through their pollination services. Bees, beetles, butterflies, and flies are some of the insects considered as pollinators (Fig. 2). There are native bees, such as the squash bee, well-adapted to local conditions benefiting not only commercial agriculture fields, but also the landscape across locations. In fact, pollinators could contribute ~\$110 million to Virginia's agricultural income annually, according to an estimation made by the Virginia Department of Agriculture and Consumer Services.

## Native Pollinator Habitats in Out-of-play Areas

Pollinator habitats can feature a variety of plants native to Virginia. These include plants that could flower throughout the spring, summer, and fall months, to ensure that there would be a constant source of pollen for the pollinators. These habitats are often undisturbed, with no irrigation, no pesticides, or without significant human intervention. These habitats act as a refuge for native species of arthropods and other animals in turfgrass systems, increasing its system biodiversity (Fig. 3 and 4).



Figure 2. From left to right Eastern carpenter bee (*Xylocopa virginica*, Hymenoptera), snowberry clearwing / hummingbird moth (*Hemaris diffinis*, Lepidoptera), and Eastern tiger swallowtail (*Papilio glaucus*, lepidoptera). Images were taken in out-of-play areas in Glen Allen, Williamsburg and Blacksburg, VA, respectively. Photos by Shannon Bradley.



Figure 3. A potential pollinator habitat at a golf course showing the variety in plant type and height in Henrico County, VA. Photo by Shannon Bradley.

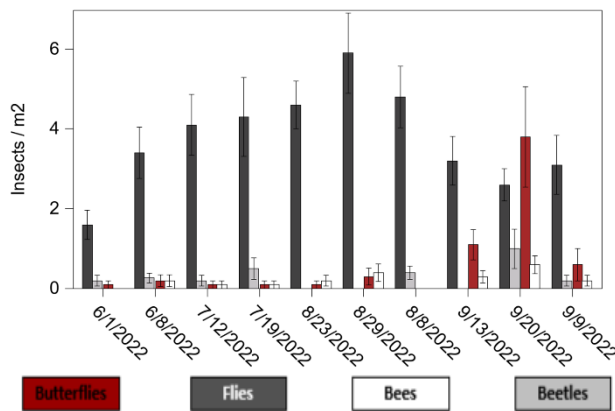


Figure 4. Counts of insects performed at an out-of-play pollinator habitat in a golf course in Williamsburg, VA, in 2022. Flies accounted for the largest group of insects that were seen interacting with flowering plants.

### Monitoring Pollinator Habitats with Light Reflectance

Out-of-play areas which can act as pollinator habitats or refuges, are not to be disturbed by excessive human intervention or maintenance. As popularity for these pollinator spaces grows, so does the need for a method of monitoring to assess habitat identification, limiting the disturbance of the area. Visual counts of pollinators, ground cover and plant categorization have been performed as part of an effort to document the relationship between these areas and pollinators (Fig. 5). Light reflectance as part of a remote sensing approach, offers a method that minimally disturbs native plants and wildlife during data collection.



Figure 5. PVC frame placed at each out-of-play pollinator habitat to mark the sampling area for assessing the presence of insect pollinators and plant type. Photo by Shannon Bradley.

### Remote Sensing

Remote sensing is the use of technology to collect information from a space that would otherwise be difficult to physically access for observation. This would include spaces that are too large to monitor or are unsafe for an individual to enter. Remote sensing includes the use of technology such as drones, sensors, cameras, and GPS; and is currently being used in nurseries and turfgrass to collect plant inventory, plant stress, and other data.

### Light Reflectance

The drones used for remote sensing can collect multispectral light reflectance data. This reflectance can include a range of visible and non-visible wavelengths such as ultraviolet (UV) and infrared (IR). Light reflectance is the measurement of the amount of light that is reflected off an object (Fig.6). The light reflectance data can be used to determine proxies associated with the health of a plant. It is expected that the healthier a plant, the higher the light reflectance is. There are some mathematical indices that can also use light reflectance data to calculate different characteristics of a plant, such as the level of green or other colors. These indices can highlight more specific light wavelengths. For instance, Normalized Difference Vegetation

Index (NDVI) is commonly used to detect the level of green in the turfgrass industry.

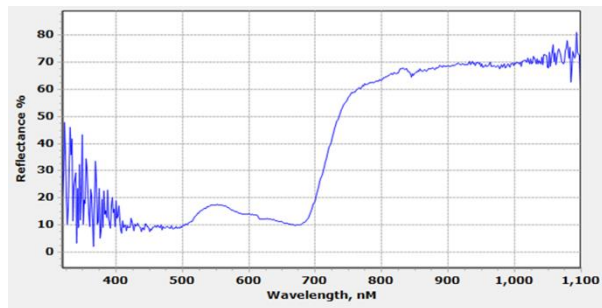


Figure 6. Light reflectance profile collected from several plants at an out-of-play area. Ultraviolet is measured from 100-400 nm, and is attractive to many pollinators. Plants with a higher percentage of UV reflectance may be likely to attract pollinators.

### **The Relationship Between Light Reflectance and Pollinators**

The light reflectance of plants could be used as a proxy to characterize the ‘quality’ of out-of-play areas on golf courses, as pollinator-friendly habitats. This reflectance is applied to several indices and then related to the number of pollinator insects that were visually scouted. An index could be used to establish the range of light reflectance present at a scouted area where high numbers of insects interacting with flowers have visited. Light reflectance indices highly correlated with the presence of pollinators could be selected to identify and locate these ‘high quality’ pollinator-friendly habitats in out-of-play areas across a golf course. Having a remote sensing approach to delimit these pollinator habitats will reduce labor and aid in improving an integrated pest and pollinator management program under golf course settings.

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## **Chapter 2: The Phenology of the Annual Bluegrass Weevil at Central and Eastern Virginia Golf Courses**

### **Abstract**

The annual bluegrass weevil (ABW, Coleoptera: Curculionidae, *Listronotus maculicollis*, Kirby) is a known pest of cool season turfgrasses in the northeast United States, being *Poa annua* (bluegrass) and *Agrostis stolonifera* (bentgrass) preferred hosts. Since the mid-2000s this pest has been found in Virginia, which is one of the southern-most states to use cool-season turfgrasses on golf courses. All stages of ABW feed on affected turfgrasses, and cost thousands of dollars to manage with insecticides such as pyrethroids and neonicotinoids. This study focused on recording the presence of adult and immature ABW at central and eastern Virginia golf courses, to determine the phenology of this pest in Henrico County, James City County, and City of Virginia Beach during 2021 and 2022. The approaches used for this monitoring effort included soap water flushes and soil core samples to determine densities of adult and immature ABW, respectively. This study determined that there were two peaks in adult densities, occurring between March and April, and June to August, depending on the location. It was also determined that there was no significance in the presence of immature ABW at the beginning of the sampling season as a predictor of the population of adult ABW, which opposes results found in northern states. The results of this study can be used by members of the turfgrass industry, especially in Virginia, to help guide the timing to deploy control tactics as well as help to understand the phenology of this pest in more southern climates.

## **Introduction**

Turfgrass is one of the largest, ground-covering crops found in Virginia, with a recorded 1.7 million acres planted as of 2004 (NASS 2006). It is reported that on average a standard 18-hole golf course can be over 150 acres of land, with approximately 100 acres being well manicured turfgrass (Lyman et al. 2007). Virginia is unique as it serves as a host to both warm- and cool-season turfgrasses due to the temperate climate. This uniqueness can lead to challenges including the outbreak of diseases and pests, requiring different treatments than in other locations. A primary turfgrass pest is the annual bluegrass weevil (ABW, Coleoptera: Curculionidae, *Listronotus maculicollis*, Kirby), which is a major concern in annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.), as both immature and adult (Kostromytska 2014). The larvae of this weevil not only feed on the bottom of the blades and leaf sheaths, but also cause browning; and their damage is unacceptable on managed turfgrass systems (Kostromytska et al. 2016). Often, damage caused by ABW larvae can be mistaken for mismanaged irrigation because the browning and patchiness caused by larvae is similar in appearance to turfgrass that is under-watered (McDonald et al. 2007). Management of this pest is costly, with golf course superintendents reportedly spending upwards of \$9,000 annually in the northeast (McGraw et al. 2017). Part of this cost is because the larvae and adults can require different and frequent treatments.

The adult ABW is a small (3.6-4.5 mm) brown or black beetle with a protruding snout, which can make it difficult to spot when monitoring at the base of infested turfgrass (Vittum 1999, McGraw et al. 2022). The adults are easy to spot when they emerge from the lower levels of the turfgrass and climb to the tops of blades of grass. The immature ABW is smaller, legless, and lighter in color than the adults, appearing as a creamy white with a brown head capsule and

measuring between 1-3 mm in length (Vittum 1999, McGraw et al. 2022). These immatures are often found in the roots or the sheath of the turfgrass, which makes locating them difficult without removing the turfgrass (McGraw et al. 2020). Adult and immature ABW can be present simultaneously in the same space during the summer months, however, only the adult ABW is expected to overwinter in nearby tree detritus (Vittum 1999, McDonald et al. 2005, Peck et al. 2007). As weather warms in late winter and early spring, the adults emerge and move throughout a manicured turfgrass space, so ABW populations can appear in different areas within the same year. The first generation of ABW is comprised of adults who overwintered and will produce the second generation of the year (Vittum 1999, Peck et a. 2007, McGraw et al. 2017).

Monitoring of both immature and adult populations is critical for managing this pest, as generations often overlap during the year (Vittum 1999, Billeisen 2017), especially as part of an integrated pest and pollinator management (IPPM) plan, which considers the needs of pollinators in a space while managing a pest. The phenology of the ABW is well understood in the northeastern parts of the United States, where it was initially identified as a pest (Cameron et al. 1968). In places like New York, the ABW typically has two generations per year, starting as early as March (Peck et al. 2007). The number of generations of ABW is dependent on the year's weather, with warmer years featuring up to three generations (Vittum 1999). In central and eastern Virginia, there is a need to further understand the phenology of this pest, to predict the emergence of the first generation, which is critical for the timing to deploy any control tactic targeting this pest.

The current methods of scouting for this pest performed by golf course personnel include soap water flushes and soil core sampling. Soap water flushes are used to monitor for adult ABW; irritated adult ABW emerge from the turfgrass when soap water is applied (Daly 2021),

with an economic injury level of 10 ABW per 0.3048 m<sup>2</sup> (McGraw et al. 2009). This method is non-destructive to turfgrass. Soil cores samples are used to monitor immature ABW populations; cores are placed under a Berlese funnel or heat lamp to extract larvae (Daly 2021). These methods were used in studies monitoring ABW phenology in western Virginia. There is currently information about ABW phenology in western Virginia, but due to geographic and climate differences, this information is likely not applicable to the entire state. The western portion of Virginia that was previously studied is in the Blue Ridge Mountain region, which features cooler temperatures. ABW have begun traveling from the cooler western portion of Virginia to the warmer coastal plain that comprises portions of central and eastern Virginia. The turfgrass industry in central and eastern Virginia needs reliable monitoring efforts for this devastating pest, especially as populations of ABW with pyrethroid resistance are reported in other states (Ramoutar et al. 2009, McGraw et al. 2017).

The main objective of this study included increasing the understanding of the phenology of ABW in central and eastern Virginia. Previous surveys of golf course superintendents conducted in 2019 (Daly 2021) suggested that there were multiple golf courses east of the Richmond area that were affected by ABW damage. These areas had not been covered in previous studies performed with the intention of documenting ABW phenology in Virginia. Therefore, there was a need for further monitoring of the insect. A secondary objective of the study was to determine if the presence and density of immature ABW in the spring could act as a predictor for the presence of adult ABW later in the season. In northern states, there is a correlation between the presence of immature ABW and adults to come. The different life stages of this pest require different management strategies, making it important to predict the presence

of the emerging adults. Understanding the phenology of this pest for the central and eastern portions of Virginia will improve management strategies of this insect on golf courses.

## **Materials and Methods**

### **In-field monitoring of adults**

Fairways and greens, measuring at least 12.1 m in length, were monitored at three locations in central and eastern Virginia, with known ABW infestations. One golf course in Henrico County and one in the City of Virginia Beach were monitored from March to November in 2021 and 2022. An additional golf course in James City County was included in 2022 and was monitored from March to November. These locations were chosen as representatives for the different regions that comprise the central and eastern portions of Virginia. Location one (Henrico County), was in a rural area, positioned at 100+ m above sea level, location two (City of Virginia Beach) was in a metropolitan area at 3 m above sea level, and location three (James City County) was in a suburban area, positioned at approximately 25 m above sea level. Four sampling points at each location were monitored every week for adults using a soap water flush (0.03 L soap: 3.79 L water), poured in a 0.3048 m<sup>2</sup> area (Koppenhöfer et al. 2020) with half poured two times, with the numbers of the adults totaled. The four sampling points were selected by proximity to potential overwintering sites, which are reported to occur in the detritus of nearby trees (Peck et al. 2007). As the season progressed, yellow turfgrass that appeared to be damaged by ABW was targeted for monitoring. Locations one (Henrico County) and three (James City County) each had one hole that was untreated, while the rest of the holes at locations one, two and three were treated. At each hole of each location, sampling points included one spot on the fairway and

approach, and two sampling points on the green. Densities of adults were noted and used to track the phenology of the ABW across seasons.

### **In-field monitoring of immatures**

Soil core samples were taken weekly at the same four sampling points where adult densities were collected. Soil core samples were taken using a metal soil core sampler (Turf-Tec International, Tallahassee, Florida; 7.62 cm diameter by 8.89 cm in length, or 3 by 3.5 inches). Due to the destructive nature of this sampling method, samples were taken after adult populations were found. Samples were removed from the soil core sampler and placed into individually marked containers and brought back to the Hampton Roads Agricultural Research and Extension Center (HRAREC), where they were kept at 4°C for less than 24 hours. Soil core samples were then quartered and placed soil-side-up under a Berlese heat lamp, where they were kept for 24 hours (Yi 2012, Daly 2021). Immature ABW and any other subterranean arthropods would crawl through the funnel, moving away from the heat, and fall into 70% ethanol. Specimen in ethanol were then observed using a stereoscope for identification. Densities and presence of the five different instars of immature ABW were noted.

### **Data analysis**

Densities of both adults and immatures, for each individual year, were subjected to a repeated measure ANOVA, using a linear mixed model (PROC MIXED, SAS, Cary, NC). The average of immature ABW and average of adult ABW were considered as the response variables. The fixed effects for both life stages were the year, location and sampling week. The sampling point, within

each location, was a random effect. Sampling week was modeled as a repeated measure, where sampling point was the subject. The Kenward-Roger correction method was used to calculate the degrees of freedom (Kenward & Roger 1997). The mean separation post ANOVAs were calculated using Tukey's HSD at  $\alpha = 0.05$ .

## **Results**

### **In-field monitoring of adults**

The variable location was significant ( $df = 2, 12.6 F = 9.72; P = 0.0028$ ); therefore, separate analyses by locations were performed. For location one (Henrico County), sampling week was a significant factor in 2021 ( $df = 23, 75.6; F = 8.04; P < 0.0001$ ) and 2022 ( $df = 32, 97.122; F = 13.68; P < 0.0001$ ), whereas year was not a significant factor in the densities of adult ABW ( $df = 1, 206; F = 0.25; P = 0.06193$ ; Fig.1). For location two (City of Virginia Beach), sampling week was a significant factor in the density of adult ABW in 2021 ( $df = 17, 57.04; F = 4.35; P < 0.0001$ ) and 2022 ( $df = 34, 163; F = 2.72; P < 0.0001$ ; Fig.2). Location three (James City County) was only sampled for one year, 2022, therefore the significance of the year could not be determined. However, sampling week had a significant effect on the adult ABW density ( $df = 35, 162; F = 4.82; P < 0.0001$ ; Fig. 3). There was also a significant interaction between the location and year for locations one (Henrico County) ( $df = 35, 38; F = 2.73; P = 0.0015$ ) and two (City of Virginia Beach) ( $df = 32, 116; F = 5.76; P < 0.0001$ ).

The largest increase in adult ABW density for location one (Henrico County) in 2021 was from the middle of July to the beginning of August (Fig. 1), and in 2022 was from the beginning of June to the end of June (Fig. 1). The largest increase in adult densities for location two (City

of Virginia Beach) in 2021 was from the middle of August to the end of August (Fig.2), and in 2022 was from the end of March to early April (Fig.2). The largest peak in adult densities for location three (James City County) occurred from the end of July to the middle of August in 2022 (Fig. 3).

There was a second largest increase in adult densities noted during each of the years. This second largest increase in adult densities usually occurred before the largest increase in adult densities, except for location one (Henrico County) in 2022. The second largest increase in adult densities for location one (Henrico County) in 2021 was from late May to early June (Fig. 1), and in 2022 was in late July (Fig. 1). The secondary increase in adult densities for location two (City of Virginia Beach) in 2021 was in late July (Fig.2), and in 2022 was from the end of May to early June (Fig.2). The secondary increase in adult densities for location three (James City County) occurred from late June to early July in 2022 (Fig. 3).

### **In-field monitoring of immatures**

There were no significant differences in the numbers of larvae across all locations, years, and sampling weeks ( $df = 2, 304$ ;  $F = 1.15$ ;  $P = 0.3191$ ). The densities of immature ABW collected by soil core samples were not sufficient for extensive analysis. There was no significance in the presence of immature ABW populations, but anecdotally, there were peaks in their densities at all locations in 2022. In 2022 at location one (Henrico County), the increased density of immatures was estimated to be 23 immatures per  $0.3048 \text{ m}^2$ . This increase in the density of immatures occurred during the third week of April (Fig. 1). In 2021 at location one (Henrico County), the increase in the density of immatures was estimated to be 5 immatures per  $0.3048 \text{ m}^2$ , which

occurred in mid-July. At location two (City of Virginia Beach), the increased density of immatures was estimated to be 24 immatures per 0.3048 m<sup>2</sup>. This increase in the densities of immatures occurred during the third week of April. In 2021 at location two (City of Virginia Beach), the increase in the density of immatures was approximately five immatures per 0.3048 m<sup>2</sup>, which occurred in early September. At location three (James City County), the increase in the densities of immatures was estimated to be 17 immatures per 0.3048 m<sup>2</sup>. This increase in the densities of immatures occurred during the third week of May (Fig. 3). The highest average density of immature ABW was found at location two (City of Virginia Beach) (Fig.2). Factors of year (df = 1; 303; F = 2.18; P = 0.1408), location (df = 2;110; F =0.30; P =0.7432), and sampling week (df = 16; 304; F = 0.46; P = 0.9644) did not have a significant effect on the average density of immature ABW.

## **Discussion**

There were two increases of adult ABW densities observed in central and eastern Virginia under golf course conditions during 2021 and 2022. The results also identified a pattern in these increases in densities, where a smaller increase in adult densities would occur four to six weeks prior to a larger increase in adult densities. In addition to documenting adult activity, an increase in the densities of immatures was found prior to the largest peak in adult densities during all these monitoring efforts. These data suggest ABW presented two in-field generations during the year in both central and eastern Virginia.

The average densities of immature ABW were not significantly different from each other when analyzed weekly, and by location and season. However, anecdotally, it appeared as though

the largest increase in density of immature ABW was documented a few weeks before the largest increase in density of adult ABW. This early presence of immatures was documented predominantly in 2022, when more soil core samples were able to be taken. Location one (Henrico County) registered the largest increase in density of immatures approximately six weeks before the largest increase in density of adults, which occurred in early to mid- June. Location two (City of Virginia Beach) documented the largest increase in the densities of immatures approximately six weeks before the largest increase in the densities of adults, which occurred in late May and early June. Location three (James City County, VA) registered the largest increase in the density of immatures approximately six weeks before the secondary increase in the density of adults in late June. This was approximately ten weeks before the largest increase in the density of adults in late July and early August. This information can be used by turfgrass professionals in eastern and central Virginia as part of their IPPM protocol.

Understanding the phenology of ABW will allow superintendents to specifically target the different generations and life stages with appropriate management tactics, such as the application of insecticides. Chemical control has been a consistent tactic used for the management of this pest; however different life stages of this pest require different controls. Adult ABW has traditionally been treated with pyrethroids (bifenthrin), organophosphates (acephate), and neonicotinoids (imidacloprid) (McGraw and Kline 2022) with some populations of adults having resistance to pyrethroids (McGraw and Koppenhofer 2009). Immature ABW populations are treated with spinosad (spinosyn), anthranilic diamides (cyantraniliprole), in addition to neonicotinoids (clothianidin) and organophosphates (trichlorfon) (McGraw and Kline 2022). The previously listed chemicals require accurate timing, and therefore understanding the life cycles of ABW can determine the most effective time for the application. The information of

the first increase in density could be used to apply immature targeting treatments. There could then be a secondary application with the second, larger increase in adult populations with the appropriate chemical controls. This could limit the number of insecticide applications needed, as professionals would be able to apply treatments as needed, therefore reducing cost and environmental impact.

In a study performed in western Virginia (Montgomery and Roanoke Counties), the densities of immature ABW were found in numbers nearing 100 at their peak (Daly 2021). The results from the present study indicated the largest peak in immature ABW found was 24 immature ABW per 0.3048 m<sup>2</sup>, approximately a quarter of the density found in Daly (2021) study. Daly (2021) also monitored adult populations using soap water flushes (Koppenhöfer et al. 2020) and found variations in adult density peaks between the locations and the different years (Table 1). Daly (2021) attributed the different density peaks to the different weather conditions experienced between the two years of the study. The current study observed different weather conditions that may have impacted the winter survival of the ABW populations and therefore the spring population. Location one (Henrico County) had a winter with lower average temperatures in 2021 and increased temperatures in 2022. Location two (City of Virginia Beach) and three (James City County) followed the same pattern as location one (Henrico County), which was a warm winter season in 2022. In 2022, there were days reaching 27° C at all the locations. Winter 2022 was milder, and therefore may have increased the survivability of the overwintering population. Increased temperatures may have also begun the first increased density in adults earlier in 2022, which could have allowed for an additional generation. The current study also found variation in ABW densities between locations, and years surveyed. Understanding the variation in populations throughout different regions, over multiple years will better assist

Virginia's turfgrass industry in individualizing their IPPM management plan to their specific region.

The results of the present study have identified a relationship between the sampling week and the density of adult ABW. This study also concluded that there was a major peak at each of the three locations and then a second peak during the same season. This would suggest that central and eastern Virginia golf courses could experience two major peaks in the adult density which equated to the presence of two generations after the overwintering generation. Some literature suggests that depending on the season, ABW can have four generations, including the population of overwintering adults emerging (Daly 2021). In previous studies performed in western Virginia, ABW were monitored from early March to early September with four peaks, which could represent four generations present, the most generations of this insect observed in a single season (Table 1). Central and eastern Virginia had three total generations, including overwintering adults, one less generation than Daly (2021) observed in western Virginia. Initially, it was thought that due to Virginia's warmer falls, compared to northeastern states, there was the potential for populations to extend well into October. Additionally, due to the coastal plain climate of the central and eastern portions of Virginia, it was thought that there may be an increase in generations due to a longer season. This was considered in the development of the protocol for the present study, where weekly sampling of adults took place well into October to ensure that if there was a late fall or early winter generation, that it would be documented. However, this study did not observe populations of adult ABW after early October, which would suggest that there is not an additional late fall generation. The populations at the peak densities were different based on the sampling location but not on the year. Location one (Henrico County, VA) registered an increase in density of adults, over 15 ABW per 0.3048 m<sup>2</sup>. This density is

unacceptable for the course, as it is above the economic injury level (McGraw et al. 2009).

Location three (James City County) featured an average of 25 adult ABW per 0.3048 m<sup>2</sup>.

Location two (City of Virginia Beach) had an average of 4 adult ABW per 0.3048 m<sup>2</sup> at the largest increase in adult population, which is below the economic injury level of 10 adult ABW per 0.3048 m (McGraw et al. 2009). Damage is likely to be visible before this threshold is met.

Regardless of location or year, a consistent pattern in adult activity was documented.

There was a smaller increase in density of adults followed by a larger increase in the density of adults four to six weeks later. This 4–6-week period seems to align with the amount of time for an ABW egg to develop into an adult (Vittum 1999). There was a variation in the timing of the major peak in adult density from June to August across locations and years. The largest peak in adult density occurred between late July and late August at all locations in 2021. In 2022, the largest peak in adult density occurred from late March to early August, with a less distinctive pattern. The second largest peak in adult densities, representing a new generation, occurred from late May to June at all studied locations in 2021. In 2022, the second largest peaks occurred from late May to late July. There was more variation between the years and a shift in date of the peak densities which may be due to differences in weather patterns in Virginia or weather patterns that deviate from northern weather. Virginia generally has a milder winter and longer summer season, than northern states. This extended summer season may explain why the ABW populations were found in such high numbers in 2022. For example, the growing degree day in based 50°F (with a biofix on March 1<sup>st</sup>) accumulation was 733 in mid-May of 2021 at location one (Henrico County), whereas on the same date in 2022, the growing degree day had already reached 826 at location one (Henrico County). This could mean that warmer weather could have shifted the emergence of the overwintering population. While immature densities might not be a precise

tool to predict the presence of adults in Virginia, there is a potential to use the emergence of the overwintering generation to determine when the largest peak of adult may occur. This proposal would benefit from additional years of observations and more experimentation.

The present study contributed data to better understand the phenology of ABW in central and eastern Virginia. There were low populations of immature ABW that limited their analysis. Low populations may be caused by the low number of soil core samples that were allowed to be taken at each golf course, due to concerns shared by participant golf course personnel. This means that a very small percentage of the course was sampled in any given week. Location three (James City County) was added after the first year of data collection to geographically fill the gap between locations one (Henrico County) and two (City of Virginia Beach), which resided over 240 kilometers apart. Location three (James City County) filled this gap and decreased the distance between locations. The limitations on destructive sampling for immatures have inspired the development of remote sensing and light reflectance, as this method may be able to monitor the health of the entire fairway, and the potential presence of ABW, without damaging the turfgrass. Further information on the use of light reflectance in the detection of ABW can be found in Chapter 3 of this thesis.

The results of this study described the phenology of ABW in eastern and central Virginia. This included the identification of two significant increases in adult density, which represent the two in-field generations of ABW that occur after the overwintered adults emerge, for a total of three generations. Eastern and central Virginia had one less generation than what was reported in a study in western Virginia (Daly 2021). Additionally, there was a pattern to the peaks in adult density identified, where a smaller peak would occur four to six weeks before a larger peak. The four to six weeks noted would represent the time for the first generation after the emergence of

overwintering adults to go through the second and larger generation. Understanding the number of generations, and when to expect those generations, is critical in the development of IPPM plans for turfgrass professionals, as it could allow them to adjust their treatments to around the increases in populations.

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**Table 1.** A comparison of the timing for adult annual bluegrass weevil peaks at different sampling sites in Virginia from 2019 to 2022. Blacksburg and Roanoke Counties are considered western and northwestern regions, respectively. Henrico County is in the north central region. James City County and the City of Virginia Beach are included in the eastern region. Adult peaks are represented by growing degree days (GDD), which is biofix 50°F starting March 1 of the listed years.

Location	Altitude (meters above sea level)	Year	Largest adult density peak (GDD)	2 <sup>nd</sup> largest adult density peak (GDD)	Reference
Montgomery County	>460	2019	1954.5	1017-1306.5	Daly (2021)
Montgomery County	>460	2020	2007.5-2327	184.5	Daly (2021)
Roanoke County	>330	2019	1228	1685.5	Daly (2021)
Roanoke County	>330	2020	2953	1302	Daly (2021)
Henrico County	>100	2021	2201-3026	431	This study
Henrico County	>100	2022	1026-1420	0-49	This study
City of Virginia Beach	3	2021	2970-3360	1866	This study
City of Virginia Beach	3	2022	1017-1361	172	This study
James City County	25	2022	2550-3133	1535-1677	This study

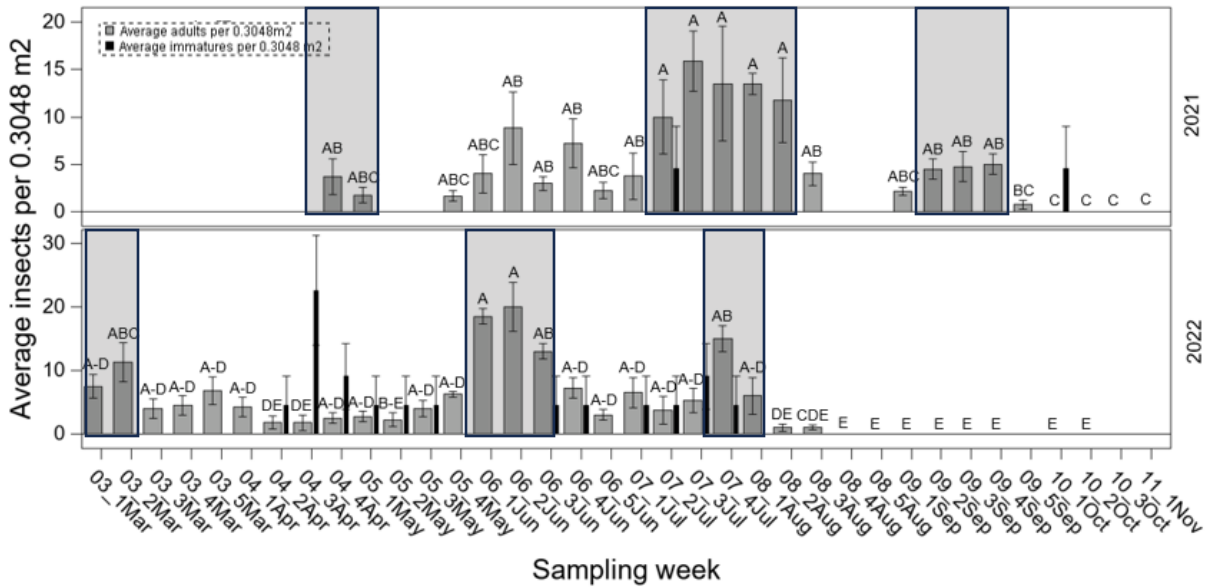


Fig. 1. Densities  $\pm$  standard error of both adults (gray bars) and immatures (black bars) for the annual bluegrass weevil collected weekly from March to November in sampling location one (Henrico County, Virginia) during 2021 (top row) and 2022 (bottom row). Bars sharing the same letters are not significantly different ( $\alpha = 0.05$ ). Shaded gray areas denote peak of insect activity.

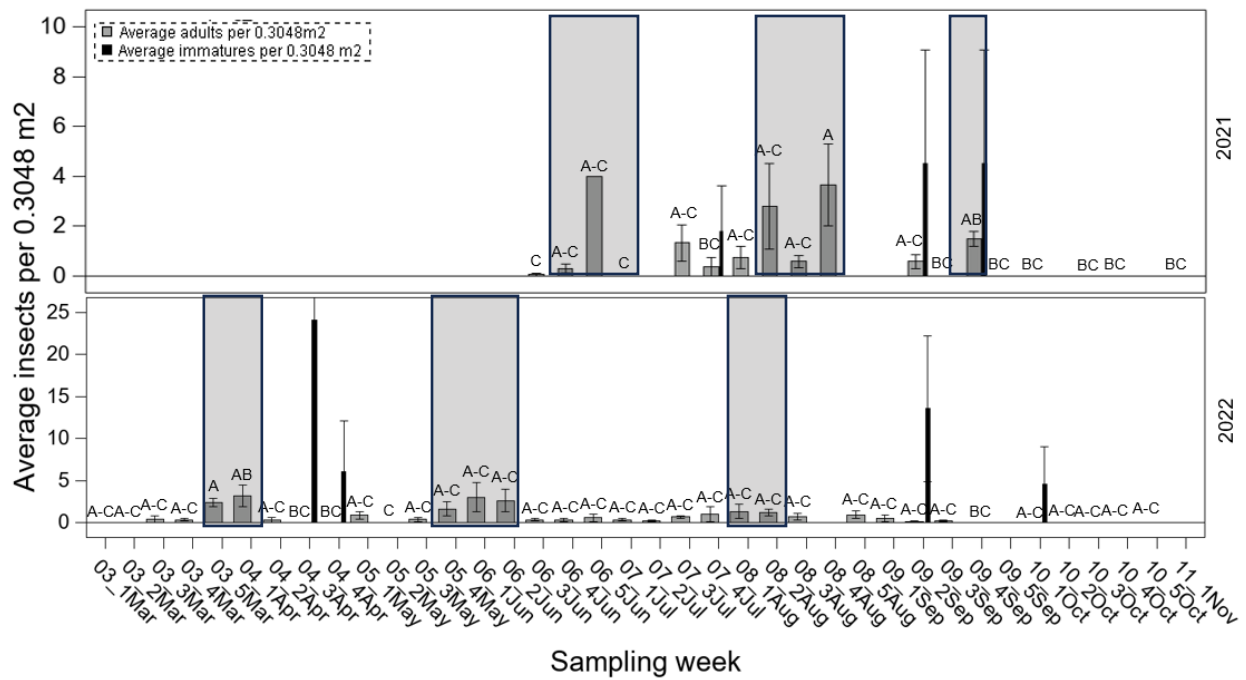


Fig. 2. The densities  $\pm$  standard error of adult (grey bars) and immature (black bars) annual bluegrass weevil populations collected weekly from March to November in sampling location two (City of Virginia Beach, Virginia). Data collected in both 2021 (top row) and 2022 (bottom row) is presented. Bars labeled with the same letters are not significantly different ( $\alpha = 0.05$ ). Location was treated both years. Shaded gray areas denote peak of insect activity.

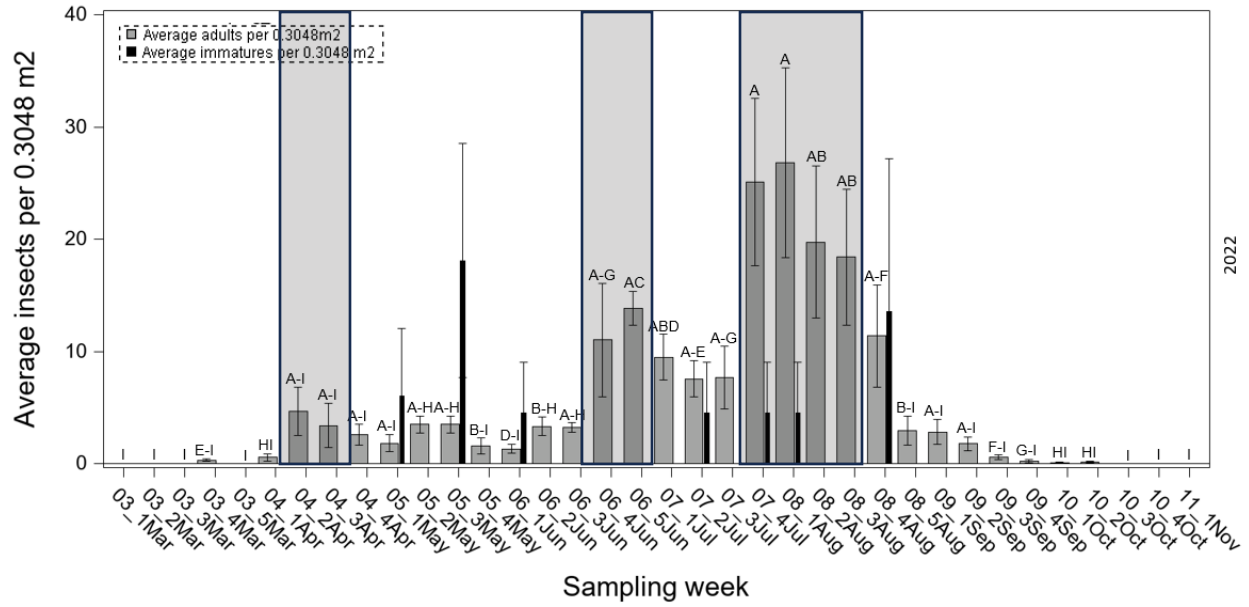


Fig.3. The densities  $\pm$  standard error of adult (grey bars) and immature (black bars) annual bluegrass weevil populations collected weekly from March to November in sampling location three (James City County, Virginia). Data was only collected in 2022 at this location. Bars labeled with the same letters are not significantly different ( $\alpha = 0.05$ ). Shaded gray areas denote peak of insect activity.

### **Chapter 3: Remote Sensing Techniques for Detection and Monitoring of the Annual Bluegrass Weevil at Virginia Golf Courses**

#### **Abstract**

The annual bluegrass weevil (*Listronotus maculicollis* Kirby, Coleoptera: Curculionidae, ABW), is an expensive pest to manage, costing Virginia golf courses over \$7,000 annually when heavily infested. Current methods for the monitoring of ABW are localized to a very small percentage of the golf course and can be destructive, which limits the ability for the monitoring of this pest over an entire fairway. Light reflectance is a tool currently used by the turfgrass industry to locate disease infected areas, but it has the potential to highlight turfgrass stressed by ABW before the turfgrass appears visually stressed. The light reflectance of turfgrass stressed by this pest in a greenhouse setting, and proximally in selected golf courses was collected during this study. The greenhouse and proximal light reflectance were collected using a hand-held spectrometer. Throughout four dates in 2022, reflectance was collected at a location in James City County, VA. This data was compared to the greenhouse reflectance collected in 2023, though there were likely additional stressors on the in-field turfgrass. Light reflectance data sets were used to calculate and compare 12 different mathematical indices. Of the indices tested, it was found that the Normalized Difference Vegetation Index (NDVI) had a consistent relationship with the presence of ABW. Based on this study, ABW and NDVI had a negative correlation. This information could be used to assist golf course superintendents in developing their integrated pest and pollinator management plan, where the use of NDVI can highlight areas of ABW stress. The timely identification of ABW ‘hot spots’ might influence the deployment of control tactics to manage this pest in golf courses.

## **Introduction**

Turfgrass is one of the most extensive ground covering crops in Virginia and requires several expenses for maintenance (NASS 2006), with a portion being spent specifically for pest control. One of the most expensive insect pests of turfgrasses in Virginia to manage is the annual bluegrass weevil (*Listronotus maculicollis* Kirby, Coleoptera: Curculionidae, ABW), costing Virginia golf courses up to \$7,000 annually, which is lower than the \$9,000 cost for golf courses in the northeast (McGraw et al. 2017). These weevils are pests at both the immature and adult life stages, as they feed on turfgrass throughout their life (Vittum 1999). Immatures ABW cause extensive damage to the roots and sheath of turfgrass, resulting in browning that can appear as a lack of irrigation (McDonald et al. 2007). Adult ABW damage the upper blades of grass (Vittum 1999, Kostromytska et al. 2016, McGraw et al. 2020), which can be less noticeable, as turfgrass is often cut regularly as part of its maintenance plan. Superintendents currently scout for this pest at their golf courses using soap water flushes on representative areas of damage, which can help them to monitor a small area of the course. ABW can be difficult to manage because control treatments differ for immatures and adults (Koppenhöfer et al. 2018), and more populations have been found with pyrethroid resistance (Ramoutar et al. 2009, McGraw et al. 2017).

Maintaining turfgrass is a labor-intensive and time-consuming process with some practices, such as the application of neonicotinoids and pyrethroids (McGraw et al. 2017), having an impact at the landscape level. Current ABW monitoring methods can be equally time-consuming. Superintendents need a method that would streamline monitoring efforts and assist them in accurately identifying areas in need of ABW management. Canopy reflectance measured remotely using drones could highlight ABW stress over a large area. Light reflectance is the measurement of the amount of light, at different wavelengths, that is reflected off an object (Liew et al. 2008). The health of a plant can be determined using light reflectance as a proxy

(Liew et al. 2008). This would allow superintendents to strategically treat only specified areas of need which could reduce the impact on pollinators, the environment, and costs. Light reflectance collected by drones is currently used by turfgrass researchers to highlight plant stress (Leinauer et al. 2014, Hamilton et al. 2009).

In previous studies, light reflectance has been used in conjunction with vegetation indices to highlight turfgrass stress, including issues with pests and irrigation (DeFauw et al. 2009, Hamilton et al. 2009, Leinauer et al. 2014). Light reflectance, using the Normalized Difference Vegetation Index (NDVI, Aparicio et al. 2000), has previously been used to highlight irrigation stress in bermudagrass (Leinauer et al. 2014). The NDVI uses red and near-infrared wavelengths to highlight stress, as a healthy plant will absorb red and near infrared wavelengths, but a stressed plant will reflect these wavelengths. Other indices, such as the Aphid Index (AI), have employed light reflectance of the actual insect specimens to predict the density of insects infesting host plants (Mirik et al. 2006a). High resolution is needed to detect individual pests, but broader resolution is acceptable for the indirect detection of insect pests.

This study aimed to determine the correlation between the presence of ABW stressed turfgrass and light reflectance. Water stress and ABW stress could appear similar for turfgrass in the field (McDonald et al. 2007). There is no previous documentation to use as a benchmark of light reflectance for turfgrass damaged by ABW. There is also a lack of data from greenhouse and controlled conditions for cool-season turfgrasses impacted by ABW, including *Poa annua* and *Agrostis stolonifera*. This study was conducted with the ultimate goal of creating light reflectance standards to detect stress induced by ABW infestation under controlled conditions. These standards then could be used to compare light reflectance collected from golf course settings.

Light reflectance has been used as a method of stress detection for turfgrass (DeFauw et al. 2009, Carrow et al. 2010, Leinauer et al. 2014, Luglio et al. 2023). Light reflectance could be a non-destructive monitoring approach (Gitelson et al. 2001) with the potential to detect ABW damage before stress would be even visible (Hamilton et al. 2009). This type of detection might allow for superintendents and turfgrass industry professionals to reduce time devoted to scouting, by only targeting areas of concern detected and identified by light reflectance.

## **Materials and Methods**

### **Initial light reflectance study**

Soil plug samples (7.62 cm diameter by 8.89 cm depth) of *Agrostis stolonifera* L. variety '007', were collected from Blacksburg, VA in June 2021. These plugs were maintained in a glass greenhouse with conditions of approximately  $28 \pm 5^\circ\text{C}$ ,  $45 \pm 10\%$  RH, and a 14:10 hr light:dark photoperiod. They were placed in turfgrass containers (D40H, Deepot Conetainers, 6.86 cm in diameter) with drainage, that were lined with cotton balls and sand, then the soil plug samples. Each soil plug sample was covered in mesh and constituted an experimental unit. For the initial portion of this study, there was one factor, ABW, with three levels. The three treatments were 0 ABW, 5 ABW, and 10 ABW, which were all collected from Blacksburg, VA. All other conditions were kept uniform. The turfgrass was cut every two weeks to a height of approximately 2.5 cm and was measured using a spectrometer (PSR- 1100F, Spectral Evolution Haverhill, MA) three times from June to July 2021. The spectrometer had a range from approximately 320- 1,100 nm, and a spectral resolution of 3.0 nm.

Initial in-field light reflectance readings were taken four times at a private golf course in James City County, VA using the same handheld spectrometer. Readings were taken between April and August 2022 at eight one m<sup>2</sup> sampling points, where the presence of adult and

immature ABW was confirmed with the use of soap water flushes (0.03 L soap: 3.79 L water), poured in a 0.3048 m<sup>2</sup> area (Koppenhöfer et al. 2020) and soil cores, respectively. Soil cores were collected with a metal soil core sampler (Turf-Tec International, Tallahassee, Florida; 7.62 cm diameter by 8.89 cm in length, or 3 by 3.5 inches). The sampling points were selected based on visual damage appearing to be ABW stress and proximity to potential overwintering locations.

### **Preparation of turfgrass samples**

Soil plug samples of *Agrostis stolonifera* (7.62 cm diameter by 8.89 cm depth), with no insecticide application made during the year, were collected from Blacksburg, VA in January 2023. These plugs were maintained in a plastic greenhouse, with approximately 28 ± 5°C, 45 ± 10% RH, and 14:10 light: dark. Within 24 hours of collection, these plugs were placed in 946.35 mL plastic deli containers with 11 drainage holes soldered into the bottom. The bottom of the container was then lined with cotton balls and filled with sand (Fig. 1). Each container constituted an experimental unit, and all containers were covered with 50 x 25 mm filament mesh per 2.54 cm<sup>2</sup>, which was then secured using rubber bands. The mesh lids were removed for biweekly fertilizer treatments (Peter's Professional 18-8-17, Marysville, OH), which were applied at half rate (0.822 kg N ha<sup>-1</sup>), and monthly full rate boscalid and pyraclostrobin for fungicide treatments (0.665 kg a.i. ha<sup>-1</sup>, Honor fungicide, BASF, Holly Springs, NC). In addition to daily monitoring, the turfgrass was cut weekly to maintain a 2.54 cm (1 inch) height. Overhead irrigation was applied twice a day for a total of 1.3 cm (0.5 inches) of water column before experiments were established.

The study was a randomized block design. There were two factors, collection date and treatment. Treatment had four levels, 0 ABW and full irrigation, 0 ABW and half irrigation, 15 ABW and full irrigation, and 15 ABW and half irrigation. Full irrigation represents 1.3 cm applied daily and 0.65 cm applied every other day. Once the treatment was applied, turfgrass was continued to be cut once a week and leaf clippings were removed. To avoid ABW loss, clippings were inspected for adults before disposal, though adults were predominately found in the soil.

### **Preparation of annual bluegrass weevil samples**

Adult ABW were collected from golf courses in Williamsburg, Richmond, and Virginia Beach, VA during March 2023. Adult collections from each location and date were kept as separate populations. These adults were extracted from the creeping bentgrass and annual bluegrass greens using lemon scent dish soap-water flushes (7.8 ml in 1 L of water) (Koppenhöfer et al, 2020). The purpose of the soap was to irritate the adult, causing them to crawl to the turfgrass surface. Adults ABW were then placed in a ventilated bucket with blades of turfgrass for transport and left in buckets for less than 24 hours. These adults were not starved to minimize the stress on them.

There was an 8:7 female to male ratio of ABW adults, confirmed by individually sorting adult ABW. The treatments contained 15 ABW, as this exceeds the threshold of 10 ABW adults per 0.3048 m<sup>2</sup>, to ensure ABW stress was detectable (McGraw et al. 2009). Adult ABW used within blocks were from the same collection date and location. Once weekly, the density of ABW adults was assessed in all experimental units, by removing the adult ABW and placing them back after a count was conducted. The density of ABW of each container was noted,

including the number of alive, dead, or missing. Every week, ABW were added to the containers as needed to ensure that the density stayed at 15 ABW.

## **Light reflectance data collection**

### ***Proximal light reflectance under greenhouse conditions***

This study established greenhouse turfgrass samples that were put under duress by both ABW and irrigation stress and collected the light reflectance of these samples. An ASD Field Spectrometer 4 with a spectral range of 350-2500 nm and a bandwidth of 1.4 nm at 350-100 nm and 1.1 nm at 1001-2500 nm (Malvern Panalytical, Malvern, UK) was used to collect the light reflectance. The pistol attachment (lens with a 5° angle) of the spectrometer was held 5.18 cm above the canopy to collect the light reflectance of a study area of 7.62 cm<sup>2</sup> (3 inch<sup>2</sup>) at each experimental unit. Samples were removed from the greenhouse during data collection, as the sun acts as the light source for the pistol attachment (Malvern Panalytical 2016). The spectrometer was calibrated using a 100% reflective white reference panel (9.92 cm Spectralon panel, Malvern Panalytical, Malvern, UK) as per the manufacturer's instructions (Malvern, UK 2016). Ten light reflectance measurements were taken from each experimental unit at each reading. These readings occurred weekly for four weeks after treatment deployment.

### ***Proximal light reflectance under field conditions***

Reflectance data was collected in 2022 at one location in James City County, VA, during four sampling dates (August 23 and 29, 2022 and September 9 and 13, 2022) at eight sampling points. Four of the sampling points were considered visually healthy and four were considered

visually stressed. The densities of adult ABW were collected and noted to confirm the presence of ABW. The areas that were sampled did not receive insecticide treatments but were fully irrigated and treated with fungicides following the program led by the participating superintendent. Adult ABW densities were collected at each of the eight sampling points. The proximal light reflectance was collected before flushes were performed at the same sampling points using an ASD Field Spectrometer 4 (Malvern Panalytical, Malvern, UK) with the pistol attachment (lens with a 5° angle), held 1 m above the sampling point, to collect an area 49 cm<sup>2</sup>. Sampling points were marked with a 1 m<sup>2</sup> PVC frame to mark the area of each point before any data collection. Ten light reflectance readings were collected at each sampling point, on each sampling date.

### **Data analysis**

Light reflectance measurements from the greenhouse and in-field collections were averaged separately using ViewSpec software (Malvern Panalytical, Malvern, UK) before being analyzed in SAS software (SAS Institute, Cary, NC) using a one-way ANOVA (PROC MIXED) to compare selected wavelengths collected from each treatment. These wavelengths were recorded from 200 to 2000 nm. There were 31 specific wavelengths selected for further analysis. These 31 wavelengths were used to calculate 12 light reflectance indices in SAS, also known as vegetation indices, used in multiple agriculture industry sectors (Table 1). Each spectral index was considered as a response variable, where treatment, collection date, and their interaction were the fix effects for the greenhouse study. Block was the sole random effect. For this study, the degrees of freedom were calculated using Kenward and Roger (1997) correction method.

Additionally, the mean separation was calculated post-ANOVA using Tukey's HSD, with  $\alpha = 0.05$ .

The relationship between selected vegetation indices and ABW adults was further analyzed using regressions (PROC REG) in SAS, with the density of ABW as the independent variable and indices as dependent variables.

## **Results**

### ***Initial light reflectance study results***

Initial studies completed in 2021 suggested that NDVI and Aphid Index (AI) were the most likely to highlight ABW stress (Table 2). NDVI and AI were the most consistent indices that were significantly different in greenhouse (NDVI:  $df = 2, 87; F=51.23; P<0.0001$ ; AI:  $df = 2, 83; F=36.01; P<0.0001$ ) and in-field light reflectance collection (NDVI:  $df = 1, 3; F=7.35; P=0.0350$ ; AI:  $df = 1, 3; F=12.45; P=0.0387$ ) (Table 2). All vegetation indices were significant in the 2021 greenhouse study except ARI ( $df = 2, 87; F= 2.15; P=0.1223$ ).

### ***Proximal light reflectance under greenhouse conditions***

The light reflectance of stressed and non-stressed turfgrass was collected seven times during a four-week period; and there was a significant difference in the NDVI values after the fifth greenhouse data collection ( $df = 3, 15; F=6.09; P=0.0064$ ) (Fig. 2). Of the other 11 indices, two were not significantly different PRI ( $df = 3, 20; F=2.26; P=0.1125$ ) and DSSI1 ( $df = 3, 15; F=3.15; P=0.0563$ ) (Table 2). The NDVI was lower in ABW- infested units than in non-infested units ( $df = 3, 15; F=6.09; P=0.0064$ ). There was no significant difference between the NDVI of the control treatment collection dates (Fig. 2).

### ***Proximal light reflectance under field conditions***

The NDVI was negatively correlated with ABW adult presence at each of four weekly data collections between August and September 2022 (Fig. 4;  $df = 1, 61$ ;  $F=13.64$ ;  $P=0.0005$ ;  $R^2 = 0.1827$ ). The regression resulted in a relationship being described as the following equation:  $NDVI = 0.7902 - 0.0142 \times ABW \text{ adult densities}$  (Fig. 4). The other 11 indices that were tested were all found to not have a significant relationship with ABW (Table 2) and therefore were not analyzed.

### **Discussion**

As previously shown by other researchers, insect pests and their associated damage can be correlated with light reflectance. In this study, there was a consistent and negative relationship between NDVI calculated from proximal light reflectance (from both greenhouse and in-field) and the presence of ABW. Overall, these studies provide evidence that the use of light reflectance may detect ABW stressed turfgrass after approximately three weeks, without apparent visual damage.

In other studies, the increased intensity of stressors was found to decrease the NDVI values for turfgrass (Leinauer et al. 2014, Caturegli et al. 2020). The negative correlation between NDVI and the presence of ABW was found to be significant after the fifth reading, 14 days post infestation, under greenhouse conditions. Readings were taken twice weekly for 4 weeks. This negative correlation over time was consistent in all treatments except the controls, which maintained a range of NDVI higher than 0.65. Changes in light reflectance after the fifth reading, 14 days post infestation, could have been originated due to female adults laying eggs in

the sheath of the turf (Vittum 1999). If eggs were laid, those eggs would have hatched and then larvae could have damaged the roots of the turf. The NDVI values from fifth reading could have represented the earliest time where damage from both adult and immature ABW could have been detected. The fifth reading would then be the earliest that the damage appeared, before it was visible. Irrigation stress of turfgrass has previously been documented as a cause for the decrease in the light reflectance of turfgrass (Leinauer et al. 2014). This was confirmed by the present study, since one experimental treatment did not contain ABW, but was stressed due to receiving half the irrigation requirement. Lower NDVI values were recorded from one of our experimental treatments, likely due to the combination of two stressors, ABW presence and lack of irrigation. All experimental units had fertilizer applied at the end of the third week, after reading six was taken. In the seventh reading, there was an increase in NDVI values in some of our experimental treatments. It was expected that NDVI values could have increased after a fertilization event, due to a plant response. The control experimental units were not under any stress (no ABW and full irrigation); and therefore, fertilizer may not have been as impactful in terms of changing light reflectance.

The trend of increased ABW populations and lower NDVI was found in proximal in-field light reflectance readings from 2022 (Fig. 4), as well as in our greenhouse studies. The golf course had full irrigation during the time this study was completed, and therefore it was not under the same duress as the treatments with half irrigation in the greenhouse study. The greenhouse study also represented a larger density of ABW than the ones found at the untreated golf course. It is likely that due to the higher presence of ABW, the greenhouse samples ended having lower NDVI values than those found in the in-field portion of this study. The NDVI

values collected in the field represented a range stress such as mowing, that was not present in the greenhouse portion of the study.

The highest number of ABW found at an in-field sampling point was eight per 0.3048 m<sup>2</sup> (26 per 1 m<sup>2</sup>) compared to 15 ABW per 0.0726 m<sup>2</sup> (207 per 1 m<sup>2</sup>) for the experimental units under the greenhouse study. The highest number of ABW found in the field was below the economic threshold of 10 ABW per 0.3048 m<sup>2</sup> (McGraw et al. 2009). Although the ABW had not reached the economic threshold, damage was still present at the collaborating golf course. The in-field portion of the study found that the lower NDVI, in association with the higher presence of ABW, had values as low as 0.63. The National Turfgrass Evaluation Program (NTEP) uses visual field assessments to assign quantitative ratings of the appearance of turfgrass that takes characteristics into consideration, such as uniformity of the turfgrass, shoot density, leaf texture, leaf orientation, smoothness, and others (NTEP 2008). This assessment rates turfgrass on a scale from one to nine, with a score of nine representing the ideal turfgrass, and a score of six representing that the minimum standards had been met (NTEP 2008). In 1999, a comparison between the NDVI of bermudagrasses and the visual field assessments were made which correlated a visual score of 9 to an NDVI of approximately 0.90 and higher, and a score of six to an NDVI of approximately 0.70 (Trenholm et al. 1999). Therefore, the acceptable NDVI value for creeping bentgrass in commercial settings is approximately 0.70, which these results fall under NTEP (2008) and Trenholm et al. (1999). Different turfgrasses have different spectral ranges and therefore, a different turfgrass may be acceptable with an NDVI below 0.70. The results from the in-field portion of this study were summarized on the following equation:  $NDVI = 0.7902 - 0.0142 \times ABW \text{ adult densities}$ . Assuming to have 10 ABW adults, the expected NDVI would be 0.65. This expected NDVI is under the accepted value of approximately 0.70 ABW and

therefore would potentially indicate to golf course superintendents that an area might be under stress from the presence of ABW. The results from future studies have the potential to highlight turfgrass under ABW stress, from aerial light reflectance. Golf course superintendents currently use drones and light reflectance to highlight other forms of stress, and therefore the need for a light reflectance threshold to detect ABW would be needed. There is even the potential for future studies to develop an index unique to damage caused by ABW or other turfgrass related insect pests.

The results of this study can act as a foundation for future development of a light reflectance approach as part of an IPPM plan. This included the identification of a negative relationship between the presence of ABW in *Agrostis stolonifera* and NDVI, as seen in the greenhouse portion of this study. Additionally, there was a negative relationship documented in the field between the presence of ABW and NDVI. The determined NDVI value that represented acceptable creeping bentgrass was proposed to be  $> 0.7$  (Trenholm et al. 1999, NTEP 2008). When the economic threshold for ABW (10 adults: McGraw et al. 2009) was applied to the equation, it yielded an NDVI of approximately 0.65, which is below the proposed NDVI value for creeping bentgrass. This information, including the proposed equation, could be used by turfgrass industry professionals to highlight areas of stress, and to target scouting to the areas of concern. The information from this study could be complemented with future research, through multiple aerial light reflectance collections, which would further benefit the turfgrass industry, as aerial drone flights would be able to potentially highlight stress over an entire course.

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**Table 1.** Light reflectance indices (vegetation indices) for various uses. This table is adapted from Luo et al. (2013). Under the ‘Formula’ column, the letter ‘R’ represents the actual light reflectance value at the indicated wavelength shown as a subscript.

<b>Spectral Index</b>	<b>Formula</b>	<b>Reference</b>
Aphid Index (AI)	$(R_{740}-R_{887})/(R_{691}-R_{698})$	Mirik et al. 2006b
Anthocyanin Reflectance Index (ARI)	$(R_{550})^{-1}/(R_{700})^{-1}$	Gitelson et al. 2001
Damage Sensitive Spectral Index (DSSI1)	$(R_{719}-R_{873}-R_{509}-R_{537})/(R_{719}-R_{873}+R_{509}-R_{537})$	Mirik et al. 2006a
Damage Sensitive Spectral Index 2 (DSSI2)	$(R_{747}-R_{901}-R_{537}-R_{572})/(R_{747}-R_{901}+R_{537}-R_{572})$	Mirik et al. 2006b
Modified Chlorophyll Absorption Reflectance Index (MCARI)	$[(R_{700}-R_{670})-0.2(R_{700}-R_{550})]*(R_{700}/R_{670})$	Daughtry et al. 2000
Narrow Band Normalized Difference Vegetation Index (NBNDVI)	$(R_{850}-R_{680})/(R_{580}+R_{680})$	Thenkabail et al. 2000
Nitrogen Reflectance Index (NRI)	$(R_{570}-R_{670})/(R_{570}-R_{670})$	Filella et al. 1995
Normalized Difference Vegetation Index (NDVI)	$(R_{850}-R_{691})/(R_{850}+R_{691})$	Aparicio et al. 2000
Photochemical Reflectance Index (PRI)	$(R_{531}-R_{570})/(R_{531}+R_{570})$	Gamon et al. 1997
Plants Senescence Reflectance Index (PSRI)	$(R_{680}-R_{500})/R_{570}$	Merzlyak et al. 1999
Structure Insensitive Pigment Index (SIPI)	$(R_{800}-R_{450})/(R_{800}-R_{680})$	Peñuelas and Inoue 1999
Triangular Vegetation Index (TVI)	$0.5[120(R_{750}-R_{550})-200(R_{670}-R_{550})]$	Broge and Leblanc 2001

**Table 2.** Analysis of Variance information for vegetation indices (see Table 1 for formulas and complete index names) calculated from data collected from the greenhouse and in-field turfgrass samples, and annual bluegrass weevil (ABW) adult densities. Bolded rows represent significant difference for a selected index with the presence of ABW adults.

Index	Year	Location	Degrees of freedom	F	P-value
<b>NDVI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>6.09</b>	<b>0.0064</b>
PRI	2023	Greenhouse	3, 20	2.26	0.1125
<b>AI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>3.36</b>	<b>0.0054</b>
<b>NRI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>6.38</b>	<b>0.0040</b>
<b>NBNDVI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>5.84</b>	<b>0.0075</b>
<b>MCARI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>6.42</b>	<b>0.0052</b>
<b>TVI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>14.11</b>	<b>0.0001</b>
DSSI1	2023	Greenhouse	3, 15	3.15	0.0563
<b>ARI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>15.47</b>	<b>&lt;0.0001</b>
<b>DSSI2</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>3.62</b>	<b>0.0380</b>
<b>PSRI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>8.82</b>	<b>0.0013</b>
<b>SIPI</b>	<b>2023</b>	<b>Greenhouse</b>	<b>3, 15</b>	<b>5.93</b>	<b>0.0071</b>
<b>NDVI</b>	<b>2022</b>	<b>In-Field</b>	<b>1, 61</b>	<b>13.64</b>	<b>0.0005</b>
PRI	2022	In-Field	1, 58	0.33	0.5685
AI	2022	In-Field	1, 61	0.19	0.6679
NRI	2022	In-Field	1, 58	0.49	0.4860
NBNDVI	2022	In-Field	1, 58.1	0.59	0.4453
MCARI	2022	In-Field	1, 58	0.00	0.9730
TVI	2022	In-Field	1, 61	0.62	0.4355
DSSI1	2022	In-Field	1, 61	0.24	0.6271
ARI	2022	In-Field	1, 58	1.39	0.2434
DSSI2	2022	In-Field	1, 61	0.39	0.5357
PSRI	2022	In-Field	1, 58	0.89	0.3482
SIPI	2022	In-Field	1, 58	0.87	0.3537
<b>NDVI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 87</b>	<b>51.23</b>	<b>&lt;0.0001</b>

<b>PRI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 87</b>	<b>18.78</b>	<b>&lt;0.0001</b>
<b>AI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 83</b>	<b>36.01</b>	<b>&lt;0.0001</b>
<b>NRI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 87</b>	<b>85.82</b>	<b>&lt;0.0001</b>
<b>NBNDVI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 87</b>	<b>49.40</b>	<b>&lt;0.0001</b>
<b>MCARI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 87</b>	<b>65.86</b>	<b>&lt;0.0001</b>
<b>TVI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 83</b>	<b>72.78</b>	<b>&lt;0.0001</b>
<b>DSSI1</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 83</b>	<b>22.51</b>	<b>&lt;0.0001</b>
ARI	2021	Greenhouse	2, 87	2.15	0.1223
<b>DSSI2</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 83</b>	<b>7.98</b>	<b>0.0007</b>
<b>PSRI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 87</b>	<b>28.19</b>	<b>&lt;0.0001</b>
<b>SIPI</b>	<b>2021</b>	<b>Greenhouse</b>	<b>2, 83</b>	<b>4.90</b>	<b>&lt;0.0001</b>
<b>NDVI</b>	<b>2021</b>	<b>In-Field</b>	<b>1, 3</b>	<b>7.35</b>	<b>0.035</b>
PRI	2021	In-Field	1, 6	0.56	0.4819
<b>AI</b>	<b>2021</b>	<b>In-Field</b>	<b>1, 3</b>	<b>12.45</b>	<b>0.0387</b>
NRI	2021	In-Field	1, 3	3.58	0.1547
NBNDVI	2021	In-Field	1, 3	3.69	0.103
MCARI	2021	In-Field	1, 3	2.35	0.2231
TVI	2021	In-Field	1, 3	3.48	0.1591
DSSI1	2021	In-Field	1, 6	3.74	0.1013
ARI	2021	In-Field	1, 3	2.75	0.196
DSSI2	2021	In-Field	1, 3	3.55	0.1559
PSRI	2021	In-Field	1, 3	3.25	0.1691
SIPI	2021	In-Field	1, 3	3.91	0.1424

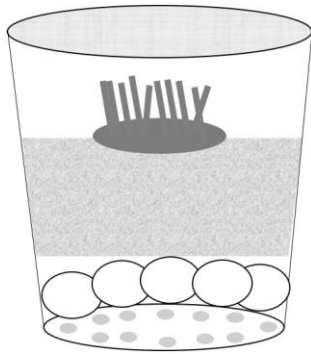


Fig. 1. Diagram showing the setup of an experimental container with 1) the mesh lid secured with a rubber band, 2) the turfgrass plug in the center, 3) sand around the plug, 4) cotton balls underneath the sand, and 5) soldered drainage holes at the bottom of the container.

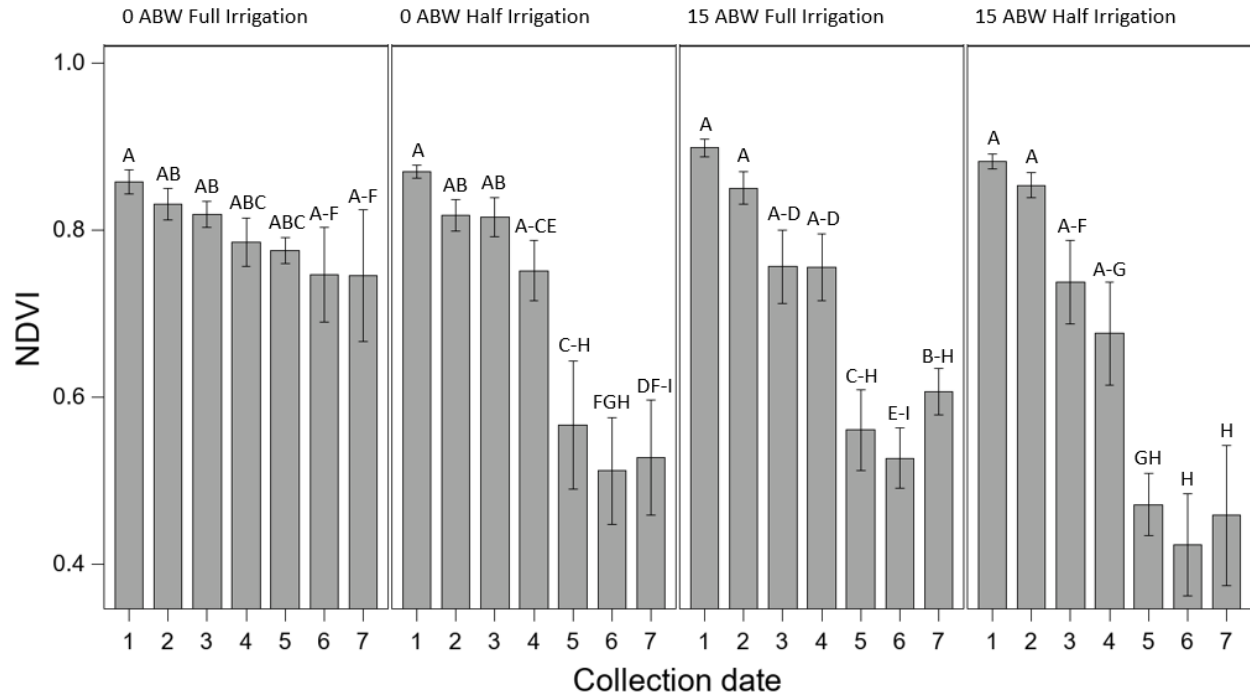


Fig. 2. Averages  $\pm$  standard error for the Normalized Difference Vegetative index (NDVI) calculated using light reflectance collected from turfgrass under greenhouse conditions from March to April 2023. Bars sharing the same letters are not significantly different ( $\alpha = 0.05$ ). The ANOVA was run across treatments and collection dates. Collection dates (X-axis) represent the light reflectance readings occurring twice a week. Vertical panels present the data for each experimental treatment.

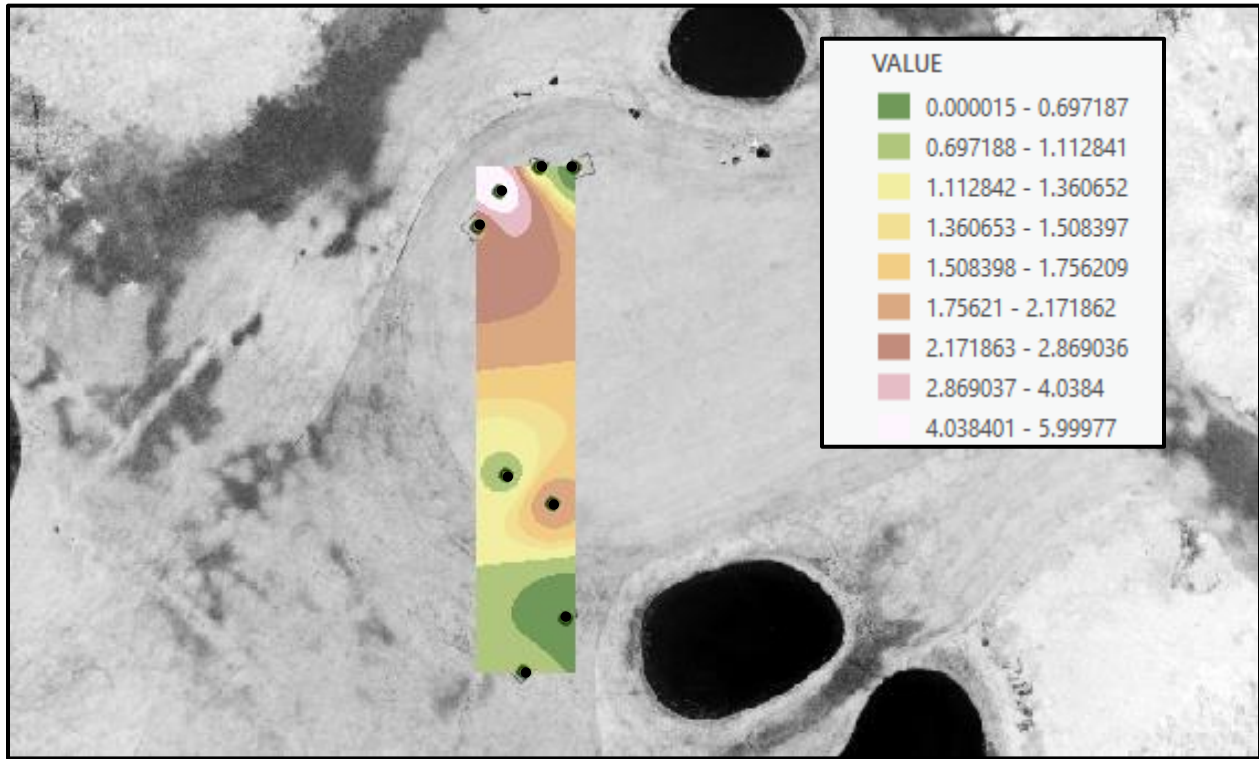


Fig. 3. Example of an inverse distance weighing map (IDW) for turfgrass infested with annual bluegrass weevil under a golf course setting. The grey background represents the values for the Normalized Difference Vegetative index, and the colorful overlay represents IDW, estimating the number of insects per  $0.3048 \text{ m}^2$ . This adult density estimation was based on data collected from in-field sampling locations (black points on the colorful map), where insect counts were performed. Data shown in this example was collected on August 23, 2022 at a golf course in Williamsburg, Virginia.



## **Chapter 4: Developing a Remote Sensing Approach to Highlight Potential Pollinator-Friendly Spaces in the Out-of-Play Areas of Virginia Golf Courses**

### **Abstract**

The decline of insect populations, including pollinators, has increased the need for refuge habitats. Out-of-play areas in golf courses are being left as low-input and low-disturbance spaces and have the potential to serve as pollinator habitats. There is limited information regarding the characteristics of these potential pollinator habitats, paired with existing pollinator populations. This study aimed to 1) catalogue existing plant canopies, by using light reflectance and other visual characteristics, and 2) document pollinator densities, at three golf courses in Virginia. Up to 30 sampling points in one out-of-play area were monitored at each golf course, where pollinator insect counts were classified to taxonomic order, canopy cover was calculated, and light reflectance was collected. Reflectance was gathered remotely and proximally, using a multispectral drone and a handheld field spectrometer, respectively. The light reflectance was used as both a proxy for plant health and to calculate indices used to determine a range of characteristics about the plants, such as chlorophyll content. This study used 13 different indices with the potential to highlight insect populations. The Structure Insensitive Pigment Index (SIPI) was found to have the most consistent correlation between out-of-play plants and the presence of potentially pollinating insects at both the aerial and proximal levels. The use of this index, as part of a remote sensing approach could highlight potential pollinator habitats in the out-of-play areas of golf courses. This approach could aid golf course superintendents as they scout for existing potential pollinator habitats and then assist them in developing their integrated pest and pollinator management plan.

## **Introduction**

In recent years, there has been a decline in insect populations recognized all over the world (Sánchez-Bayo and Wyckhuys 2019, Kremen et al. 2002). In addition, it has been estimated approximately 40% of insects would be facing potential extinction (Sánchez-Bayo and Wyckhuys 2019), in part due to habitat loss (Vitousek et al. 1997). Included in this decrease in population of insects has been the decrease in pollinators (Kremen et al. 2002). Insect pollinators are animals that aid in plant reproduction through the distribution and dispersal of pollen. Pollinating insects include some bees (Order Hymenoptera), beetles (Coleoptera), butterflies (Lepidoptera), and flies (Diptera) (Ollerton 2017). Pollinators are attributed to facilitating approximately 87.5% of plant reproduction worldwide (Ollerton et al. 2011). These animals provide pollination, a critical ecological service that also benefits the economy, when related to agricultural crops. For instance, it is estimated that pollinators contribute approximately \$55 million annually to Virginia's economy (VDAC 2010).

This decline in insects, including pollinating insects, has been in part attributed to loss of habitat (Sánchez-Bayo and Wyckhuys 2019). Loss of habitat can be caused by introduction of invasive species or rising temperatures, but most of the habitat loss is caused by land development (Sánchez-Bayo and Wyckhuys 2019). Habitat loss has led to calls for action in developing spaces for pollinators and the exploration of alternative habitats (Shepherd & The Xerces Society 2002). One of the unexpected locations for conservation of wildlife has included golf courses, which were previously considered 'green deserts' (Barton 2008). Golf courses were considered green deserts because they were seen as a monoculture that did not provide the spatial structure for the diverse needs of existing fauna.

Golf courses span thousands of acres in the US, but up to 70% of golf course acreage is non-playable, non-managed space featuring plants that break up the monoculture of turfgrass (Warnken et al. 2001). These non-playable areas include vast expanses of trees, native plants, and the potential to host insects facing habitat loss. While golf courses modify the natural habitats, they have a higher ecological value than other human developed lands, which makes them more attractive to wildlife (Colding and Folke 2009). The out-of-play areas of golf courses have already been recognized as places preserving populations of pollinating animals such as insects and birds (Brennan 1992, Merola-Zwartjes et al. 2005, Gross and Eckenrode 2012). The recognition of these out-of-play areas as potential wildlife habitats has led to continued conservation efforts on golf courses (Gross and Eckenrode 2012, Dale et al. 2020). Leaving the out-of-play areas of golf courses untreated means that scouting for pests will not be necessary in this area, which reduces chemicals in the environment, and materials and labor costs. Additionally, leaving these areas untreated can allow for the presence of natural predators of turfgrass pests (Billeisen et al. 2021). The presence of out-of-play areas therefore creates a mutually beneficial relationship between the golf course system and the pollinators. Efforts such as Operation Pollinator, the USDA's Conservation Cover (Code 327), and numerous from the Xerces Society include planting native plants, minimally disturbing existing out-of-play areas and implementing an integrated pest and pollinator management plan (IPPM).

An IPPM is the development and administration of a plan that manages pests and pest damage, while also considering the needs of pollinators (Lundin et al. 2021). Tactics for an IPPM plan can include synched mowing and insecticide applications, reduced use of broad-spectrum insecticides, and the use of biological control methods (Lundin et al. 2021). A key feature of an IPPM plan is scouting for disease and insect pests before applying pesticides, to ensure pest

populations are present before treatment methods are deployed (Lundin et al. 2021). The use of drones and light reflectance in the turfgrass industry has become a method of monitoring for a variety of diseases and pests, such as the annual bluegrass weevil, as discussed in Chapters 2 and 3. These methods have the potential to extend past the fairway into the out-of-play areas to monitor for potential pollinator habitats.

Drones offer the ability to collect data from large areas of land, including spaces that may not be accessible to humans, and would decrease the potential for disturbing wildlife in those spaces of interest (Jiménez López and Mulero-Pázmány 2019). In addition to being a proxy for plant health, light reflectance has been used to monitor flowers and the attraction of pollinators to flowers (Whitney et al. 2009, Koski et al. 2013). Collected wavelengths from selected areas can be combined in mathematical light reflectance indices, which can be used to highlight individual characteristics of a plant, such as the chlorophyll content or the potential for blooming (Peñuelas and Inoue 1999). For example, the structure insensitive pigment index (SIPI) uses blue and orange wavelengths to monitor plant health and yield, such as the yield capability of flowering plants (Peñuelas and Inoue 1999).

Despite the importance of these out-of-play areas on Virginia golf courses, there is still a lack of information cataloging the benefit of these spaces. The purpose of this study was 1) to document the flora and insect populations of the out-of-play areas of Virginia golf courses, and 2) to develop an approach using light reflectance to detect potential pollinator spaces based on specified indices. There has been a need to catalogue what populations of insects could be present in the out-of-play areas to determine if there would be pollinators among the insects present. The development of an approach using light reflectance to detect potential pollinator habitats would assist golf course superintendents in the development of their IPPM plans. With

this approach, superintendents would be able to identify and then scout specified areas of interest to ensure that they would be proactively looking to preserve pollinator-friendly habitats in their courses. There is also a need to compare the catalogued insect data to the light reflectance approach to validate the correlation between insects and indices.

## **Materials and methods**

### **Selection of out-of-play areas**

Three golf courses in central and eastern Virginia were selected for this study with two being chosen for the 2022 season and a third being added in the 2023 season. These golf course locations were selected because of their already existing un-managed areas as part of out-of-play areas, near maintained turfgrass, with dimensions of at least 30 m long by 20 m wide. The selected golf courses were in Henrico County (Location one, years 2022 and 2023), James City County (Location two, years 2022 and 2023), and New Kent County (Location three, only 2022). Locations one and two were private courses, while location three was a public course. Each experimental area was mowed once during the winter before monitoring occurred, as per direction from the Xerces Society in native upkeep (Shepherd and The Xerces Society 2002) but did not receive any further intervention from the golf course personnel. In 2022, 15 1m<sup>2</sup> sampling points were monitored in each experimental area, however this increased to 30 sampling points in 2023 to account for the size of the out-of-play area. The sampling points, which were marked with numbered metal flags, were spaced 3.96 m apart to account for 1m<sup>2</sup> monitoring frames that were placed at each flag (Dale et al. 2020, Palmersheim 2022). Flags remained for the duration of the study and acted as a guide for the frames placed for observation of flora and insects. The GPS coordinates of each flag were taken using an Emlid Reach Rover

and Base (Emlid, Budapest, Hungary). GPS coordinates were taken to act as ground control points and to accurately place count data.

### **Ground monitoring of insects and plant canopy characteristics**

In 2022, the insect densities were collected nine times between May and September at locations one and two. Insect densities were recorded four times between April and May in 2023 at all locations. The decrease in monitoring dates between 2022 and 2023 was due to logistic issues. Each of the sampling points were bordered with the frames and then observed for one minute by one observer. During the observation time, insects that interacted with or landed on flowering plants within the frame were counted. Specifically, Diptera, Hymenoptera (only bees), Lepidoptera and Coleoptera were counted and classified to order, and visually classified to family when possible. Insects that were within the frame but not interacting with a flowering plant were not counted, as it was less likely that they would be pollinators.

Several plant canopy characteristics were also recorded at each of the four sampling dates in 2022 and 2023. Plant canopies were assessed only within the frames. Observations included noting the percentage of the total frame that was covered by all the canopy (percent ground coverage). The living foliage within the frame was then classified as flowering or non-flowering, and non-flowering was then further classified to broad leaf or grasses (Evans and Love 1957). Flowering foliage included both the flowers and foliage from observed plants. The visible colors of flowering plants inside the monitoring frame were also noted including white, violet, blue, green, yellow, orange, red, and brown flowers. One sample of each type of flowering plant that was found within the frames was collected, pressed between paper, and identified. Once identified, types of flowering plants were also noted.

## **Light reflectance data collection**

The four density recording dates at each location corresponded with light reflectance data collection in 2023, and four of the density recording dates at locations one and two corresponded with light reflectance data collection. Light reflectance was collected proximally using the ASD Field Spectrometer 4 (Malvern Panalytical, Malvern, UK) with the pistol attachment lens with a 5-degree angle. The pistol attachment was held facing the foliage at a 90-degree angle, one meter above the ground for each sampling point, based on an equation provided by the instrument's manufacturer. The sun acts as the light source for this spectrometer attachment, and therefore measurements were taken as close to solar noon as possible and on sunny days. The spectrometer was calibrated before each use with a 100% reflective white reflectance panel (9.92 cm Spectralon panel, Malvern Panalytical, Malvern, UK), as per the manufacturer's instructions (Malvern Panalytical 2016). Ten measurements were taken at each sampling point during each sampling date and were then averaged later in the laboratory.

Light reflectance was also collected aerially using the DJI Phantom 4 Multispectral drone (DJI, Da-Jiang Innovations, Shenzhen, China), connected to a RTK GPS base (Malvern Panalytical, Malvern, UK). Before each flight, the drone was calibrated using a 6X Multispectral Reflectance Panel (Sentera, Saint Paul, Minnesota). Flights were flown between 35 m and 50 m depending on the tree line at each of the courses and had a 75% frontal overlap. The sampling point frames were left in place for the flights, to act as ground control points. Each location had one flight plan created through the DJI Go application (DJI, Da-Jiang Innovations, Shenzhen, China) to ensure the same flight path for each of the four sampling dates. Due to the distance between locations and weather, the locations could not all be monitored on the same day. Monitoring of each location was completed within three days of each other.

## Light reflectance data processing

The proximal light reflectance measurements were averaged for each of the sampling points on each date, using the software associated with the spectrometer instrument, ViewSpec software (Malvern Panalytical, Malvern, UK). Thirteen mathematical indices were calculated in SAS software (SAS Institute, Cary, NC; Table 1) using light reflectance data.

Aerial light reflectance data was stitched together as an orthomosaic layer in Pix4D (Pix4D, Prilly, Switzerland) and then selected tile bands were imported into ArcGIS (Esri, Redlands, California), with the GPS for sampling points and count data. The drone collected five bands of color including blue, green, red, near infrared (NIR) and red edge. In ArcGIS, five different mathematical indices (Table 2) were calculated using ArcGIS software's 'Calculator' function (Esri 2021). These indices were direct translations of indices seen in Table 1, or band specific indices, using NV5Geospatial's conversion chart (2023). The calculator function allows for mathematical equations using the color bands to be calculated and assigns a numerical value to each pixel based on the imagery within each band. The out-of-play areas were then isolated using the 'Mask' function, which allows for the information within a specified polygon to exclusively be used (Fig.1).

The count and GPS data were merged and used to generate an inverse distance weighing map (IDW, Fig.2). The IDW extrapolation estimated the presence of insects based on known populations from the surrounding known data points. More specifically, IDW is an interpolation that estimates data point values based on their proximity to data points with known values, with estimates that are closer to known values being more influenced by their presence (Esri, 2021). The IDW method of interpolation was selected due to the ability of defining the parameters and selected known points (Esri, 2023). The IDW map was then sectioned into 0.3048 m<sup>2</sup> sections

using the Fishnet function in ArcGIS, which segments a map while assigning labels. These labeled sections are then used in generating the 'Zonal Statistics' for both the individual indices and the density of insects (Fig.3). These values were then extracted for further analysis.

## **Data analysis**

Insect density data from all data collection dates were compared using a general linear mixed model (PROC MIXED, SAS Institute, Cary, NC). Densities of different insect groups were dependent variables, where location by year was the sole fixed effect. The location and year were combined to make one variable, as not all the locations were monitored in both years. Date by location and year were considered as random effects. The degrees of freedom were calculated using the Kenward-Roger correction method (Kenward and Roger 1997) and the mean separation post-ANOVA was calculated by using Tukey's HSD at  $\alpha = 0.05$ .

Data from plant characteristics was correlated with the presence of insects, using a generalized linear model (PROC GLM SELECT). The model included factors such as the percent of ground cover, proportion of grasses vs. broad leaf plants, proportion of flowering and non-flowering plants, and the colors present (white, violet, blue, green, yellow, orange, red, brown). Additionally, both proximal (ASD) and aerial (drone-based) light reflectance measurements (dependent variable) were analyzed separately using regressions (PROC REG) with density of insects as the independent variable. Each of the model factors, such as ground cover, was then compared to the density of insects (PROC MIXED).

## **Results**

## Ground monitoring of insects and plant canopy characteristics

There was not a significant difference in the densities of bees ( $df = 4, 10; F = 1.24; P = 0.3560$ ) or flies ( $df=4, 10; F=0.13; P=0.9675$ ) between locations by year. There was a significant difference for both the number of beetles ( $df= 4, 10; F=11.29; P =0.0010$ ) and butterflies ( $df= 4, 10; F=7.71; P =0.0042$ ) for location by years. There appeared to be a peak in insect densities in the first week of August 2022 and the fourth week of April 2023 at location one (Henrico County) (Fig. 4). At location two the peak in insect densities appeared to occur in the fifth week of August 2022, and the second week of April 2023 (Fig. 5). Location three had a peak in the second week of April 2023 (Fig. 6).

Percentage of broadleaf plants, percentage of ground cover, presence of yellow, and percentage of flowering canopy were the most likely factors to predict the presence of pollinating insects (listed here from most likely to least likely). There was a significant difference on pollinating insects between the locations by year for percentage of broadleaf ( $df=4, 24.3; F =55.54; P <0.0001$ ), percentage of ground cover ( $df=4, 25; F =19.33; P <0.0001$ ), presence of yellow flowers ( $df=4, 21; F =11.60; P <0.0001$ ).

There was not a significant difference for pollinating insects when the percentage of the canopy that was flowering was analyzed at the locations by year ( $df=4, 23.8; F =0.96; P =0.4475$ ). The families of bees noted in this study included Apidae, Halictidae and Andrenidae, which are recognized as pollinators (Texas Tech University 2023). The families of beetles that were recorded included Coccinellidae, Cantharidae, Elateridae; where some members of the family Cantharidae have been recognized as pollinators (Texas Tech University 2023). The families of butterflies that were recorded during this study included Lycaenidae (superfamily Papilionoidea), Pieridae, Danainae (superfamily Nymphalidae), and Hesperidae. Some of the

families of butterflies that contain pollinators include Papilionidea, Pieridae, Nymphalidae (Texas Tech University 2023). The families of flies that were recorded in this study included Syrphidae, Asilidae, and Sarcophagidae; with Syrphidae being one of the families of flies included as pollinators (Texas Tech University 2023).

The flowering plants that were present during the study included butterfly milkweed (*Asclepias tuberosa*, Asclepiadaceae), common milkweed (*Asclepias syriaca*, Asclepiadaceae), swamp milkweed (*Asclepias incarnata*, Asclepiadaceae), dandelions (*Taraxacum* spp., Asteraceae), buttercups (*Ranunculus bulbosus*, Ranunculaceae), creeping violets (*Viola sororia*, Violaceae), henbit (*Lamium amplexicaule*, Lamiaceae), chickweed (*Stellaria media*, Caryophyllaceae), white clover (*Trifolium repens*, Fabaceae), and red clover (*Trifolium pratense*, Fabaceae). All plants, excluding the different species of milkweed, were present at all locations in 2023. Swamp milkweed was found at location one (Henrico County, common milkweed was found at location two (James City County), butterfly milkweed was found at location three (New Kent County). Dandelions and white clovers were found at all locations in both 2022 and 2023.

### **Proximal light reflectance of out-of-play areas**

Proximal light reflectance calculated indices had different correlations with the densities of insects based on the location (Table 3). Of the 13 indices tested, there were eight indices that were found to be statistically significant at each of the locations and when data from all three locations was averaged (Table 3). Of the eight indices, Photochemical Reflectance Index (SIPI) and Plants Senescence Reflectance Index (PSRI) were two indices with high  $R^2$  values ( $> 0.045$ ;

Fig. 8, only for individual locations). And consistently, there was a negative correlation between both SIPI and PSRI, and the density of insects (Table 3).

### **Aerial light reflectance of out-of-play areas**

There were five light reflectance indices calculated based on aerial light reflectance data. SIPI had a negative correlation with the total number of insects (Fig. 9;  $df = 1, 756$ ;  $R^2 = 0.1717$ ;  $P < 0.0001$ ), bees (Fig. 9;  $df = 1, 756$ ;  $R^2 = 0.3060$ ,  $P < 0.0001$ ), beetles ( $df = 1, 756$ ;  $R^2 = 0.0340$ ,  $P < 0.0001$ ), and flies (Fig. 9;  $df = 1, 756$ ;  $R^2 = 0.1719$ ,  $P < 0.0001$ ), but had a positive relationship with butterflies (Table 4;  $df = 1, 756$ ;  $R^2 = 0.0378$ ,  $P < 0.0001$ ). PSRI- B (blue) consistently had a negative correlation with the total number of insects ( $df = 1, 756$ ;  $R^2 = 0.3722$ ,  $P < 0.0001$ ), bees ( $df = 1, 756$ ;  $R^2 = 0.3808$ ,  $P < 0.0001$ ), beetles ( $df = 1, 756$ ;  $R^2 = 0.0340$ ,  $P < 0.0001$ ), butterflies ( $df = 1, 756$ ;  $R^2 = 0.0163$ ,  $P = 0.0004$ ), and flies ( $df = 1, 756$ ;  $R^2 = 0.3349$ ,  $P < 0.0001$ ) (Table 4). On average, SIPI had a higher  $R^2$  than PSRI.

### **Discussion**

Consistently, there were insects interacting with flowers at each of the monitoring dates, regardless of year or location, in the out-of-play areas at golf courses. These insects were present regardless of the percentage of flowering plants documented. Correlating plant composition and light reflectance could be used as a proxy to predict the presence of insects interacting with flowering plants in out-of-play areas. In this study, there was a negative correlation found between the index SIPI and the presence of insects found at both the proximal and the remote level of light reflectance collection.

The presence of insects in these out-of-play areas (acting as potential pollinator habitats) was recorded from April to September, independent of the year. This would indicate that insects are present throughout the year, as is consistent with work established by Adamson et al. (2012), which denotes the presence of bees pollinating in Virginia. Insects interacting with the flowers of plants within the sampling points were exclusively counted; insects must interact with the flower of a plant for pollination to occur.

Due to the lack of herbicides applied to these out-of-play areas weeds, such as some of the previously listed plants were able to grow. Weeds including clover and milkweed have been cited as being a food source for pollinating insects such as bees (Lundin et al. 2021). While some of the plants found in this study are turfgrass weeds, they could provide flowers for insects to interact with and cost nothing to manage. The presence of insects from families known to pollinate in these areas aligned with the proposed slogan “if you build it, they will come” (O’Brien and Arathi 2020). Plants in the families of Asclepiadaceae, Asteraceae, and Lamiaceae are in the seed mix planted in Billeisen et al. (2021) specifically to attract pollinator communities in managed turfgrass systems. These families were found growing naturally in the out-of-play areas of the locations for the current study.

This study showed a negative correlation between the presence of insects and SIPI. The wavelengths used in the SIPI formula include ones in the visible range of blue and orange, as well as NIR and is used to predict plant yield (Peñuelas and Inoue 1999). Additionally, PSRI was found to have a negative relationship with the presence of insects; and used red, NIR, and blue or green wavelengths (Merzlyak et al. 1999). In the aerial light reflectance analysis, it was found that the adjusted formula of PSRI that used the blue band in place of green had a stronger relationship with the presence of insects. Both indices that had the highest correlation with the

presence of insects included wavelengths or bands in the blue and NIR range. Insects within the same order generally can see similar wavelengths due to the presence of photoreceptors that can see in the ultraviolet (UV), blue, and green range (Briscoe and Chittka 2001). Hymenoptera (bees), Coleoptera (beetles), and Diptera (flies) all see within the UV, blue, and green range (Skorupski and Chittka 2010, Lin and Wu 1992, Wilson et al. 2021). Lepidoptera (butterflies) can also see UV, blue, and green, but they also have some additional receptors that allow for red to be seen as well (Arikawa et al. 1987). Due to their minimal range of sight, insects are attracted to these colors, especially UV. The use of blue as a band and wavelength range in both indices resulted in the highest correlation to insect presence. This strong relationship may be due to the inclusion of colors visible to these insects. The indices that highlighted exclusively the red range and NIR range, such as the Normalized Difference Vegetative Index (NDVI), were not as successful to correlate with the presence of insects, as indices that included the blue band.

An index that used wavelength within the UV may have been more successful in correlating the presence of insects, due to their strong preference for wavelengths within this range. The ASD Field Spectrometer had the ability to collect data within the UV range, but the DJI Phantom 4 Multispectral drone could not collect data within this range. Indices were limited to fall within the capabilities of the equipment. There are cameras and additional pieces of equipment that can be included on the drone to add the ability to collect light reflectance within the UV range. The addition of such materials may be beneficial in future studies to be able to test indices that include UV or develop a unique index for the detection of pollinators. Regardless of the cameras on the drone, more flights at additional locations are needed to further catalogue the out-of-play areas at Virginia golf courses. This data only represents golf courses of central and

eastern Virginia, but this work could be expanded to characterize the out-of-play areas of other regions.

The results of this study can be used as the foundation for the development of light reflectance techniques to highlight potential pollinator habitats under golf course conditions. The consistent presence of insects throughout all locations, from April to September, shows that they were present in the out-of-play areas of golf courses. Some of the insects that were recorded included those in families with the potential to pollinate; and they were found on plants that have no management cost. The aerial and proximal light reflectance of these plants resulted in a negative correlation with SIPI, which uses wavelengths within the blue range, bands that are visible to insects. Golf courses can benefit from the knowledge of the insects recorded during this study and their relationship with SIPI, as this information allows for the use of light reflectance to monitor potential pollinator habitats. Understanding where there may be populations of pollinators can allow golf course personnel to adjust their IPPM plan to suit the needs of those pollinators.

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**Table 1.** Light reflectance indices with various uses in agriculture. This table is adapted from Luo et al. (2013). Under the ‘Formula’ column, the letter ‘R’ represents the actual light reflectance value at the indicated wavelength shown as a subscript.

<b>Spectral index</b>	<b>Formula</b>	<b>Reference</b>
Aphid Index (AI)	$(R_{740}-R_{887})/(R_{691}-R_{698})$	Mirik et al. 2006a
Anthocyanin Reflectance Index (ARI)	$(R_{550})^{-1}/(R_{700})^{-1}$	Gitelson et al. 2001
Damage Sensitive Spectral Index (DSSI1)	$(R_{719}-R_{873}-R_{509}-R_{537})/(R_{719}-R_{873}+R_{509}-R_{537})$	Mirik et al. 2006b
Damage Sensitive Spectral Index 2 (DSSI2)	$(R_{747}-R_{901}-R_{537}-R_{572})/(R_{747}-R_{901}+R_{537}-R_{572})$	Mirik et al. 2006a
Modified Chlorophyll Absorption Reflectance Index (MCARI)	$[(R_{700}-R_{670})-0.2(R_{700}-R_{550})] * (R_{700}/R_{670})$	Daughtry et al. 2000
Narrow Band Normalized Difference Vegetation Index (NBNDVI)	$(R_{850}-R_{680})/(R_{580}+R_{680})$	Thenkabail et al. 2000
Nitrogen Reflectance Index (NRI)	$(R_{570}-R_{670})/(R_{570}-R_{670})$	Filella et al. 1995
Normalized Difference Vegetation Index (NDVI)	$(R_{850}-R_{691})/(R_{850}+R_{691})$	Aparicio et al. 2000
Photochemical Reflectance Index (PRI)	$(R_{531}-R_{570})/(R_{531}+R_{570})$	Gamon et al. 1997
Plants Senescence Reflectance Index (PSRI)	$(R_{680}-R_{500})/R_{570}$	Merzlyak et al. 1999
Structure Insensitive Pigment Index (SIPI)	$(R_{800}-R_{450})/(R_{800}-R_{680})$	Peñuelas and Inoue 1999
Triangular Vegetation Index (TVI)	$0.5[120(R_{750}-R_{550})-200(R_{670}-R_{550})]$	Broge and Leblanc 2001

**Table 2.** Light reflectance indices (vegetation indices) adapted and used to only process aerial light reflectance data. Indices with an asterisk are adaptations of the ones presented in Table 1, to account for the color bands collected with the drone. Under the ‘Formula’ column, the letter ‘R’ represents the actual light reflectance value at the indicated wavelength shown as a subscript. NIR represents the Near-Infrared band.

Spectral index	Formula	Adjusted formula	References
Structure Insensitive Pigment Index (SIPI)	$(R_{800}-R_{450})/(R_{800}-R_{680})$	* $(\text{NIR}-\text{Blue})/(\text{NIR}-\text{Red})$	Peñuelas and Inoue 1999
Plants Senescence Reflectance Index – Blue (PSRI-B)	$(R_{680}-R_{500})/R_{570}$	* $(\text{Red}-\text{Blue})/ \text{NIR}$	Merzlyak et al. 1999
Plants Senescence Reflectance Index – Green (PSRI-G)	$(R_{680}-R_{500})/R_{570}$	* $(\text{Red}-\text{Green})/ \text{NIR}$	Merzlyak et al. 1999
Green Leaf Index (GLI)	$(\text{Green}-\text{Red}) + [(\text{Green}-\text{Blue}) / (\text{Green} \times 2)] + \text{Red} + \text{Blue}$		Louhaichi et al. 2001
Green Chlorophyll Index (GCI)	$(\text{pNIR} / \text{pGreen}) - 1$	* $(\text{NIR} / \text{Green}) - 1$	Gitelson et al. 2003

**Table 3.** Equations, degrees of freedom, and P-values generated from the linear regressions between the total number of pollinating insects, represented by (insect), and individual light reflectance indices (see Table 1 for complete index names) calculated from proximal light reflectance data. Bolded rows denote the model was significant.

Location	Equation	Degrees of freedom	P-value
<b>Henrico County</b>	<b>SIPI = 1.2153 - 0.0304 × (insect)</b>	<b>1, 252</b>	<b>0.0001</b>
<b>Henrico County</b>	<b>PSRI = 0.0997 - 0.0105 × (insect)</b>	<b>1, 252</b>	<b>0.0007</b>
<b>Henrico County</b>	<b>DSSI2 = 1.4934 - 0.0166 × (insect)</b>	<b>1, 252</b>	<b>0.0096</b>
<b>Henrico County</b>	<b>ARI = 3.0737 + 0.1645 × (insect)</b>	<b>1, 252</b>	<b>0.0026</b>
<b>Henrico County</b>	<b>DSSI1 = 1.5503 - 0.038 × (insect)</b>	<b>1, 252</b>	<b>0.0020</b>
Henrico County	TVI = 17.021 - 0.1107 × (insect)	1, 252	0.6558
Henrico County	MCARI = 0.087 + 0.0008 × (insect)	1, 252	0.5838
<b>Henrico County</b>	<b>NBNDVI = 0.6868 + 0.0169 × (insect)</b>	<b>1, 252</b>	<b>0.0034</b>
<b>Henrico County</b>	<b>NRI = 0.1374 + 0.0114 × (insect)</b>	<b>1, 252</b>	<b>0.0411</b>
<b>Henrico County</b>	<b>AI = 5.2962 - 0.1595 × (insect)</b>	<b>1, 252</b>	<b>0.0022</b>
Henrico County	PRI = -0.0747 - 0.0004 × (insect)	1, 252	0.4793
<b>Henrico County</b>	<b>NDVI = 0.6677 + 0.018 × (insect)</b>	<b>1, 252</b>	<b>0.0037</b>
<b>James City County</b>	<b>SIPI = 1.1452 - 0.018 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>PSRI = 0.0648 - 0.0073 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>DSSI2 = 1.4375 - 0.0125 × (insect)</b>	<b>1, 345</b>	<b>0.0008</b>
<b>James City County</b>	<b>ARI = 1.9733 - 0.0757 × (insect)</b>	<b>1, 345</b>	<b>0.0231</b>
<b>James City County</b>	<b>DSSI1 = 1.4423 - 0.028 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>TVI = 20.888 + 0.7413 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>MCARI = 0.1154 + 0.0084 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>NBNDVI = 0.7559 + 0.0146 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>NRI = 0.2368 + 0.0165 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>AI = 5.3464 - 0.1686 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>James City County</b>	<b>PRI = -0.0782 - 0.0013 × (insect)</b>	<b>1, 345</b>	<b>0.0002</b>
<b>James City County</b>	<b>NDVI = 0.7423 + 0.0156 × (insect)</b>	<b>1, 345</b>	<b>&lt;0.0001</b>
<b>New Kent County</b>	<b>SIPI = 1.1791 - 0.0457 × (insect)</b>	<b>1, 238</b>	<b>0.0005</b>
<b>New Kent County</b>	<b>PSRI = 0.0782 - 0.0171 × (insect)</b>	<b>1, 238</b>	<b>0.0005</b>
<b>New Kent County</b>	<b>DSSI2 = 1.4941 - 0.0347 × (insect)</b>	<b>1, 238</b>	<b>0.0005</b>
New Kent County	ARI = 2.1202 - 0.1421 × (insect)	1, 238	0.0672
<b>New Kent County</b>	<b>DSSI1 = 1.5122 - 0.0699 × (insect)</b>	<b>1, 238</b>	<b>0.0005</b>
New Kent County	TVI = 22.246 + 0.749 × (insect)	1, 238	0.1697
New Kent County	MCARI = 0.1379 + 0.0054 × (insect)	1, 238	0.2662
<b>New Kent County</b>	<b>NBNDVI = 0.7241 + 0.0331 × (insect)</b>	<b>1, 238</b>	<b>0.0004</b>
<b>New Kent County</b>	<b>NRI = 0.2105 + 0.341 × (insect)</b>	<b>1, 238</b>	<b>0.0008</b>

<b>New Kent County</b>	<b>AI = 4.6063 - 0.141 × (insect)</b>	<b>1, 238</b>	<b>0.0008</b>
New Kent County	PRI = -0.0819 - 0.0021 × (insect)	1, 238	0.1022
<b>New Kent County</b>	<b>NDVI = 0.7087 + 0.0354 × (insect)</b>	<b>1, 238</b>	<b>0.0004</b>
<b>All Locations</b>	<b>SIPI = 1.116 - 0.0122 × (insect)</b>	<b>1, 599</b>	<b>&lt;0.0001</b>
<b>All Locations</b>	<b>PSRI = 0.0563 - 0.005 × (insect)</b>	<b>1, 599</b>	<b>&lt;0.0001</b>
<b>All Locations</b>	<b>DSSI2 = 1.4245 - 0.0089 × (insect)</b>	<b>1, 599</b>	<b>0.0012</b>
All Locations	ARI = 2.2434 - 0.0153 × (insect)	1, 599	0.6479
<b>All Locations</b>	<b>DSSI1 = 1.3935 - 0.0173 × (insect)</b>	<b>1, 599</b>	<b>0.0004</b>
<b>All Locations</b>	<b>TVI = 21.993 + 0.1648 × (insect)</b>	<b>1, 599</b>	<b>0.0004</b>
All Locations	MCARI = 0.1309 + 0.0027 × (insect)	1, 599	0.0601
<b>All Locations</b>	<b>NBNDVI = 0.7734 + 0.0093 × (insect)</b>	<b>1, 599</b>	<b>0.0002</b>
<b>All Locations</b>	<b>NRI = 0.25 + 0.0091 × (insect)</b>	<b>1, 599</b>	<b>0.0033</b>
<b>All Locations</b>	<b>AI= 4.7687 - 0.0683 × (insect)</b>	<b>1, 599</b>	<b>0.0407</b>
All Locations	PRI = -0.0819 - 0.0004 × (insect)	1, 599	0.2581
<b>All Locations</b>	<b>NDVI = 0.7608 + 0.0099 × (insect)</b>	<b>1, 599</b>	<b>0.0002</b>

**Table 4.** Equations, degrees of freedom, and P-values generated from the linear regressions between the total number of insects and individual taxa groups, represented by (insect), and individual light reflectance indices (see Table 2 for complete index names) calculated from aerial light reflectance data. Bolded rows denote the model was significant.

Spectral index	Dependent variable	Equation	Degrees of freedom	P-value
<b>SIPI</b>	<b>Total insects</b>	<b>SIPI = 1.4391 - 0.0673 × (insects)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-B</b>	<b>Total insects</b>	<b>PSRI = 0.1714 - 0.0363 × (insects)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-G</b>	<b>Total insects</b>	<b>PSRI = 0.0133 - 0.015 × (insects)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>GLI</b>	<b>Total insects</b>	<b>GLI = 0.153 - 0.025 × (insects)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>GCI</b>	<b>Total insects</b>	<b>GCI = 0.7017 - 0.0426 × (insects)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>SIPI</b>	<b>Bees</b>	<b>SIPI = 1.4154 - 0.2365 × (bees)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-B</b>	<b>Bees</b>	<b>PSRI-B = 0.1508 - 0.09767 × (bees)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-G</b>	<b>Bees</b>	<b>PSRI-G = 0.0008 - 0.0242 × (bees)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
GLI	Bees	GLI = 0.1257 - 0.0184 × (bees)	1, 756	0.1023
<b>GCI</b>	<b>Bees</b>	<b>GCI = 0.6781 - 0.1158 × (bees)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>SIPI</b>	<b>Beetles</b>	<b>SIPI = 1.340 - 0.1164 × (beetles)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-B</b>	<b>Beetles</b>	<b>PSRI-B = 0.1241 + 0.0168 × (beetles)</b>	<b>1, 756</b>	<b>0.0463</b>
PSRI-G	Beetles	PSRI-G = -0.0057 + 0.0022 × (beetles)	1, 756	0.6609
GLI	Beetles	GLI = 0.1209 + 0.0007 × (beetles)	1, 756	0.9642
<b>GCI</b>	<b>Beetles</b>	<b>GCI = 0.6779 - 0.2125 × (beetles)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>SIPI</b>	<b>Butterflies</b>	<b>SIPI = 1.3384 + 0.2928 × (butterflies)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-B</b>	<b>Butterflies</b>	<b>PSRI-B = 0.1305 - 0.0704 × (butterflies)</b>	<b>1, 756</b>	<b>0.0004</b>
<b>PSRI-G</b>	<b>Butterflies</b>	<b>PSRI-G = 0.0012 - 0.1112 × (butterflies)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
GLI	Butterflies	GLI = 0.1244 - 0.0571 × (butterflies)	1, 756	0.1507
<b>GCI</b>	<b>Butterflies</b>	<b>GCI = 0.6763 - 0.4655 × (butterflies)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>SIPI</b>	<b>Fly</b>	<b>SIPI = 1.4387 - 0.1033 × (flies)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-B</b>	<b>Fly</b>	<b>PSRI-B = 0.1688 - 0.529 × (flies)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>PSRI-G</b>	<b>Fly</b>	<b>PSRI-G = 0.0141 - 0.0242 × (flies)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
<b>GLI</b>	<b>Fly</b>	<b>GLI = 0.1615 - 0.0504 × (flies)</b>	<b>1, 756</b>	<b>&lt;0.0001</b>
GCI	Fly	GCI = 0.6624 - 0.0165 × (flies)	1, 756	0.1726

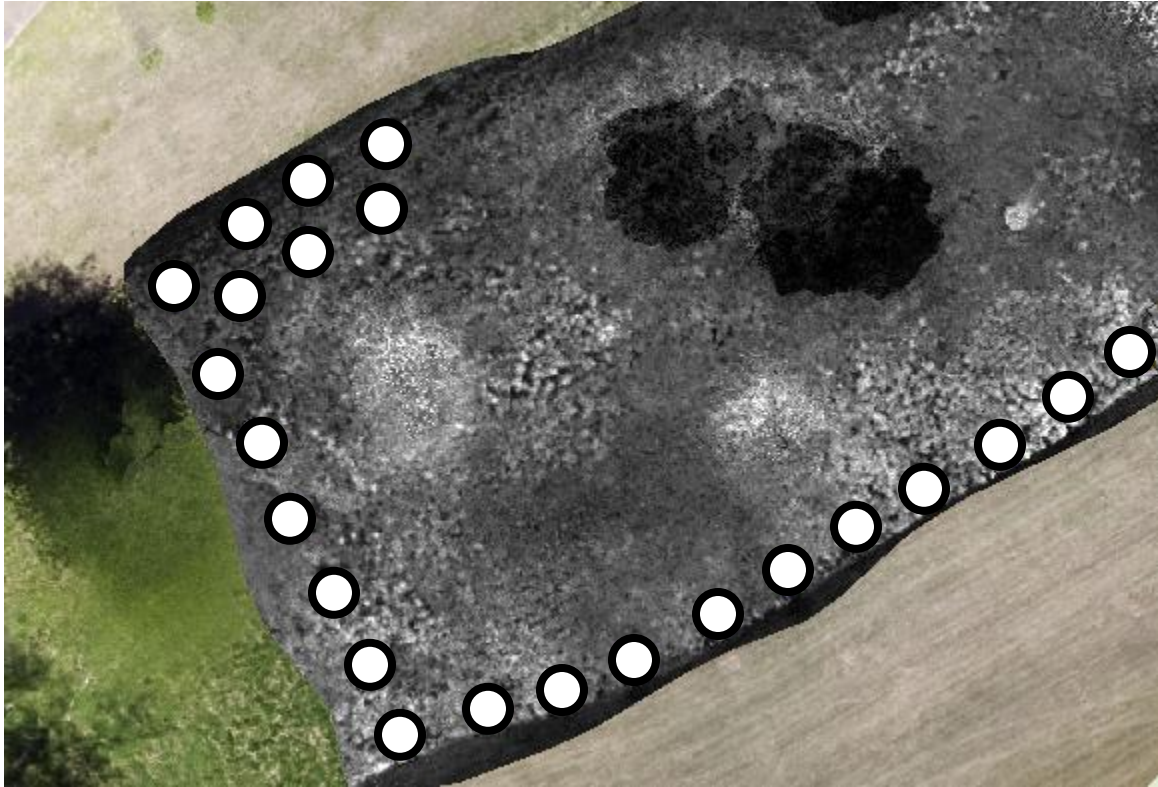


Fig.1 Example of a light reflectance index layer loaded in ArcGIS as an orthomosaic for an out-of-play area at a golf course. The colorful background represents the images that were collected and combined to create the orthomosaic map. The grey tone layer represents the values for the Green Chlorophyll Index. The density of insects was based on data collected from in-field sampling locations (black and white circles), where insect counts were performed. Data shown in this example was collected on May 11, 2023, at a golf course in Williamsburg, Virginia.

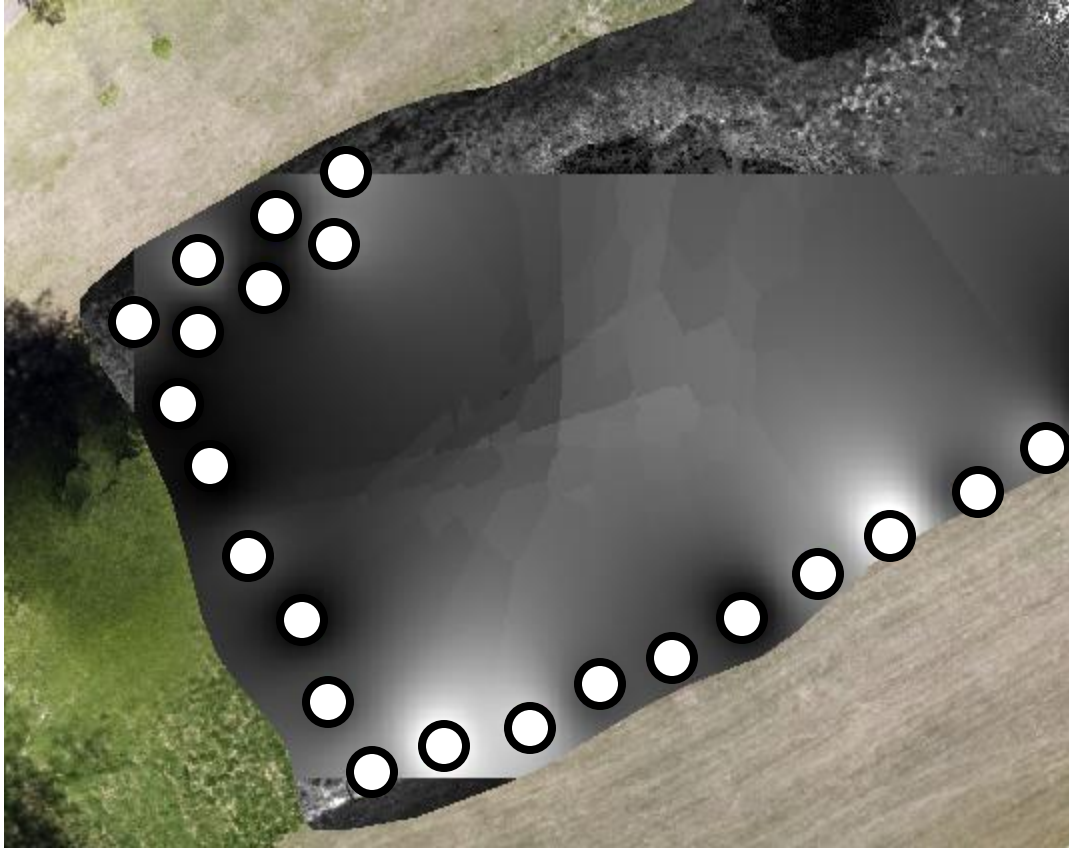


Fig. 2. Example of an inverse distance weighing map (smooth grey tone layer), layered on an index layer (textured grey layer) and the orthomosaic map (colorful base-layer). The textured grey tone layer represents the values for the Green Chlorophyll Index. The density of insects was based on data collected from in-field sampling locations (black and white circles), where insect counts were performed. Data shown in this example was collected on May 11, 2023, at a golf course in Williamsburg, Virginia.

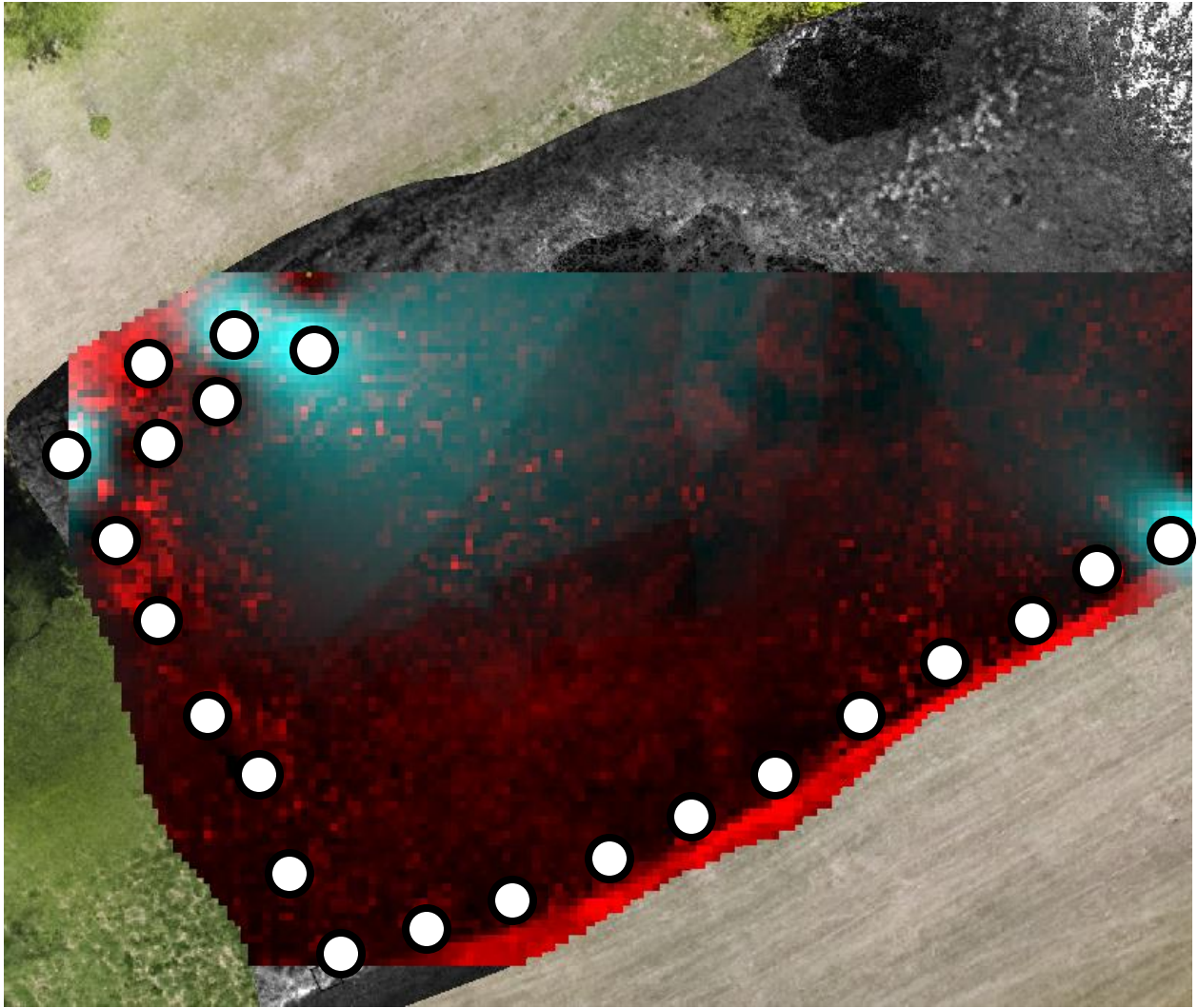


Fig. 3. Example of composite bands (red and blue layer), which creates a visual relationship between the index layer (textured grey toned layer) and the IDW (smooth grey toned layer). The colorful background represents the images that were collected and combined to create the orthomosaic map. The textured grey tone layer represents the values for the Green Chlorophyll Index. The density of insects was based on data collected from in-field sampling locations (black and white circles), where insect counts were performed. Data shown in this example was collected on May 11, 2023, at a golf course in Williamsburg, Virginia.

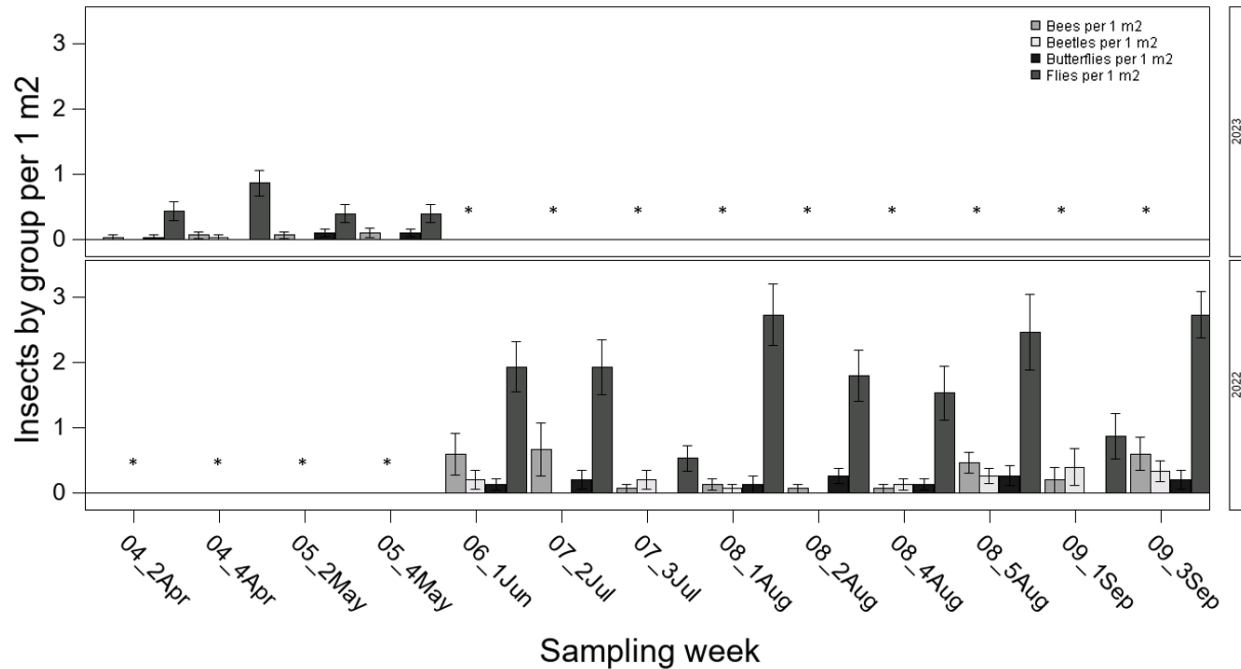


Fig. 4. Densities  $\pm$  standard error of bees (light grey bars), beetles (white bars), butterflies (black bars), and flies (dark grey bars) for the insects monitored from April to September in sampling location one (Henrico County, VA). The top row represents 2023 and the bottom row represents 2022. There was no significant difference between each group over the listed dates. Asterisks represent no data collected on a sampling week. The label for the X-axis denotes month number and the week numbers within a month.

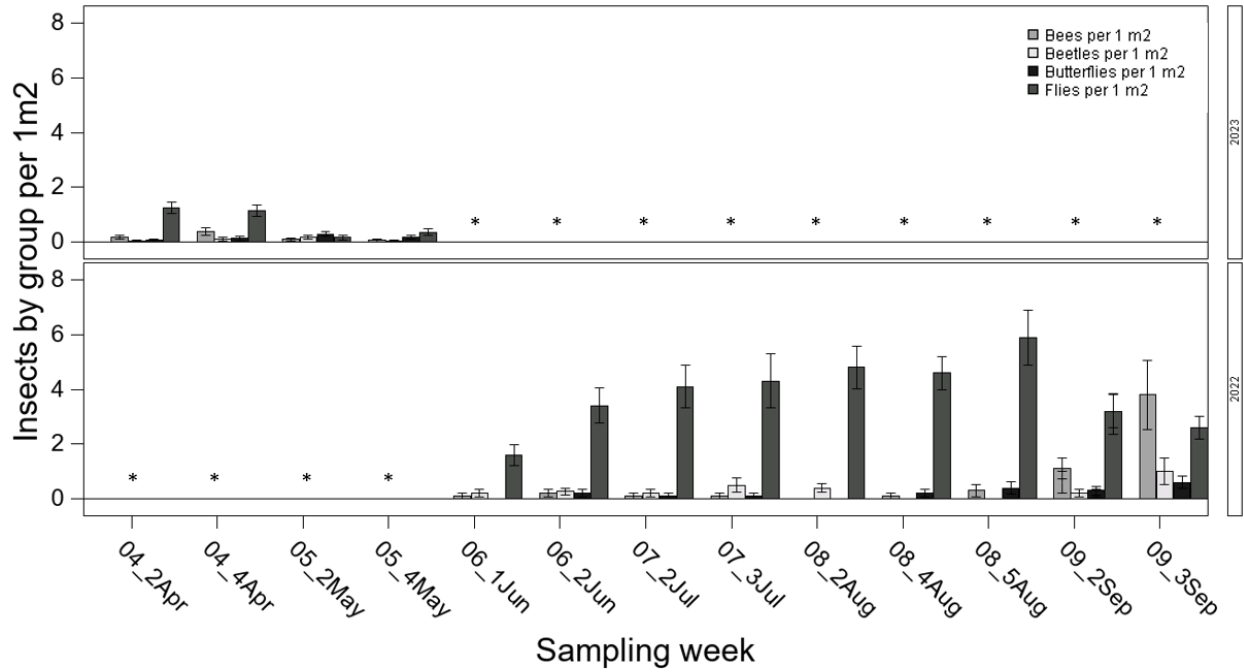


Fig. 5. Densities  $\pm$  standard error of bees (light grey bars), beetles (white bars), butterflies (black bars), and flies (dark grey bars) for the insects monitored from April to September in sampling location two (James City County, VA). The top row represents 2023 and the bottom row represents 2022. There was no significant difference between each group over the listed dates. Asterisks represent no data collected on a sampling week. The label for the X-axis denotes month number and the week numbers within a month.

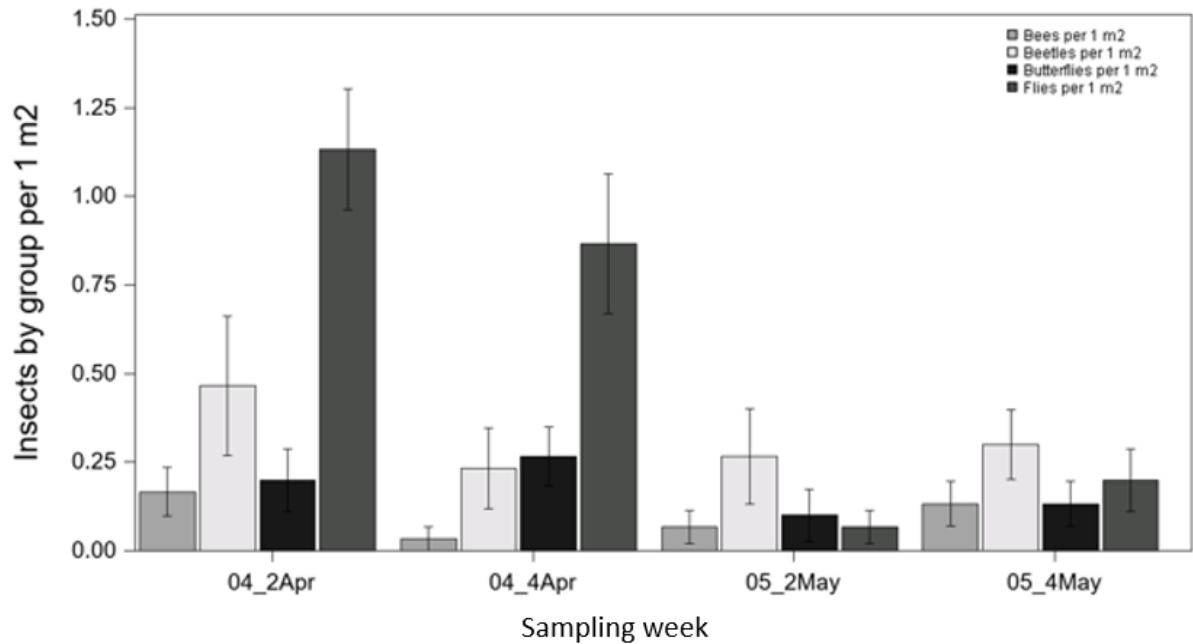


Fig. 6. Densities  $\pm$  standard error of bees (light grey bars), beetles (white bars), butterflies (black bars), and flies (dark grey bars) for the insects monitored from April to May in 2023 from sampling location three (New Kent County, VA). There was no significant difference between each group over the listed dates. The label for the X-axis denotes month number and the week numbers within a month.

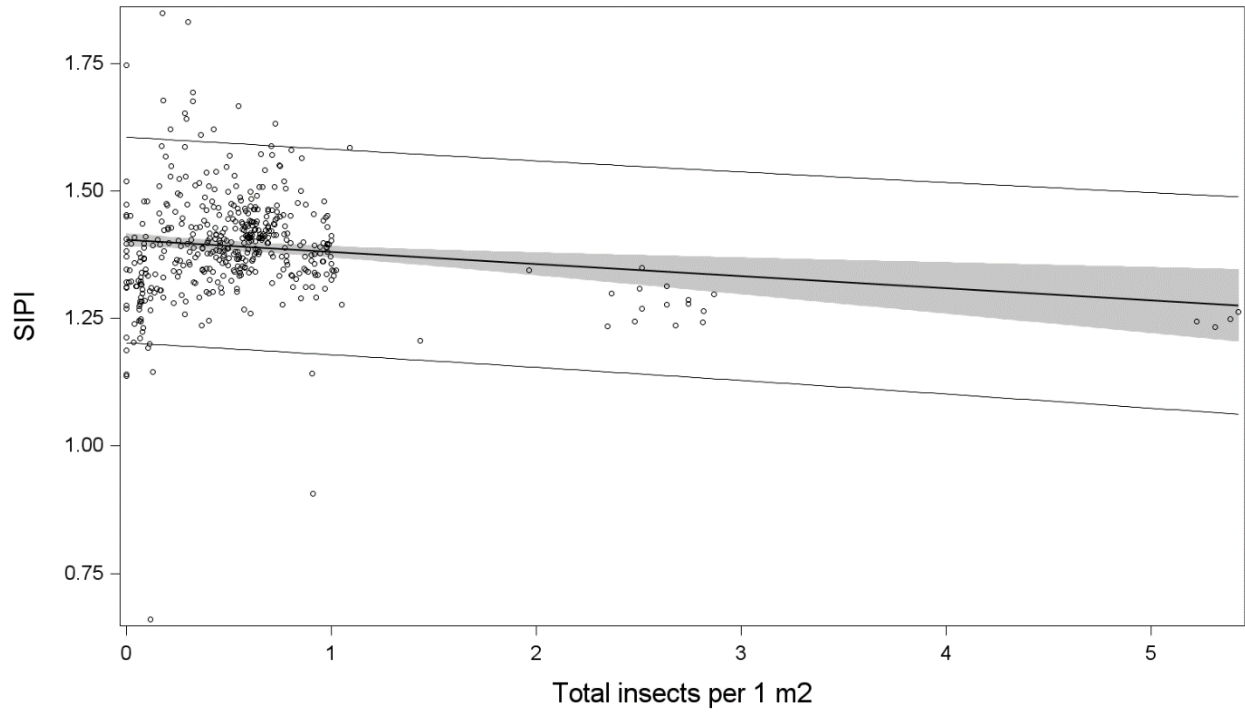


Fig. 7. Scatter plot showing one of the linear regressions performed correlating a light reflectance index (Y-axis) and pollinating insect densities (X-axis). Insect densities were collected at location one (Henrico County, VA) and the Structure Insensitive Pigment Index (SIPI) was calculated with aurally collected light reflectance data. Data shown represents both 2022 and 2023.

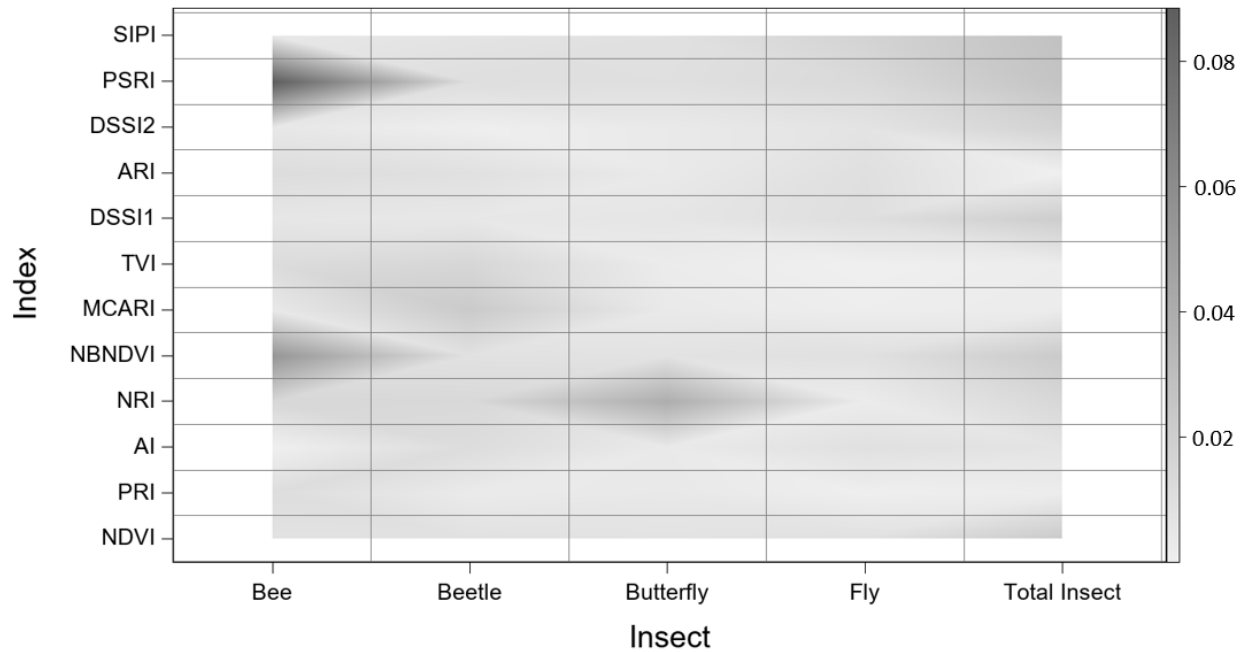


Fig.8. Contour plot showing the relationship between each individual index (Y-axis) and insect type (X-axis). The grey scale color represents the  $R^2$  value for each regression. The darker the shade of grey inside the plot, the higher the value of  $R^2$ . Total insect represents the sum of all the other insect types compared to the average of the corresponding index. Proximal light reflectance values from all collection dates and locations were used to generate this contour plot.

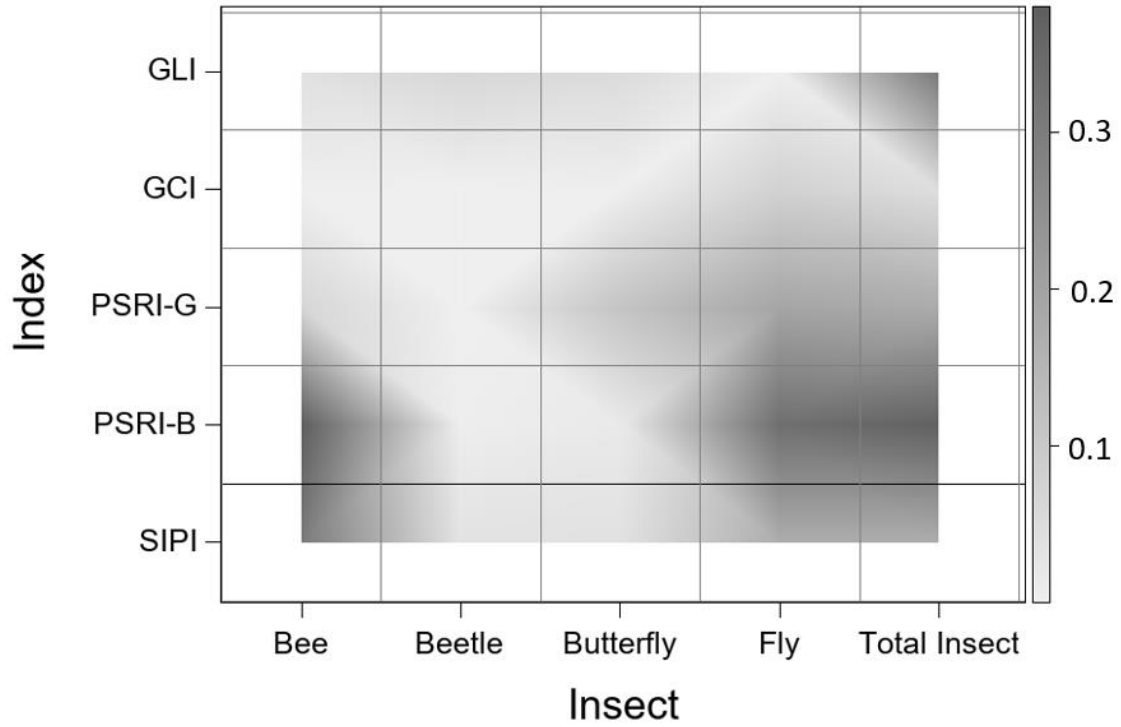


Fig. 9. Contour plot showing the relationship between each individual index (Y-axis) and insect type (X-axis). The grey scale color represents the  $R^2$  value for each regression. The darker the shade of grey inside the plot, the higher the value of  $R^2$ . Total insect represents the sum of all the other insect types compared to the average of the corresponding index. Aerial light reflectance values averaged from all collection dates and locations were used to generate this contour plot.