**ASSESSING GENETIC DIVERSITY OF CLARIREEDIA MONTEITHIANA IN GEORGIA TURFGRASSES AND EXPLORING NOVEL DOLLAR SPOT MANAGEMENT STRATEGIES**

by

WILLIS T. SPRATLING

(Under the Direction of Bochra A. Bahri and Alfredo D. Martinez-Espinoza)

ABSTRACT

Dollar spot, caused by *Clarireedia* spp., is one of the most problematic diseases of turfgrass worldwide. It diminishes functional and aesthetic quality of several turfgrass species by causing foliar blighting. Little is known about *Clarireedia* population dynamics in the southeastern U.S. Additionally, conventional control strategies, particularly repetitive fungicide applications, are costly and cause fungicide resistance issues. This work explores population structure and genetic diversity of *C. monteithiana* in Georgia turfgrasses and investigates the efficacy of UV-C radiation and oxygenated/ozonated nanobubble technology in controlling the disease. A total of 210 dollar spot isolates were obtained from various turfgrass hosts and locations throughout Georgia from 2019 to 2023, and *C. monteithiana* was identified as the most prevalent causal agent of dollar spot in the state. Genotyping-by-sequencing of 149 *C. monteithiana* isolates revealed population structure and genetic variability within the species through the detection of two genetic populations, both of which appear to reproduce clonally and evolve primarily through mutation. These findings emphasize the need for ongoing monitoring of *C. monteithiana* populations, as more diverse pathogens are better able to overcome common

management strategies. Regarding UV-C radiation efficacy against dollar spot, daily low-dose applications significantly reduced pathogen mycelial growth and disease severity in *in vitro* and growth chamber settings, respectively. In field trials, a novel autonomous delivery system was used to administer UV-C treatments, resulting in significant reductions in disease severity over two growing seasons. Although UV-C was tested only against dollar spot in seashore paspalum, these results warrant further exploration of its effects against disease in other turfgrass species. In contrast, oxygenated and ozonated nanobubble water spray applications did not reduce dollar spot severity across multiple growth chamber and field trials, likely due to gaseous loss during application. Improving overhead spray technology to prevent this loss is likely necessary for these treatments to be effective in turfgrass. Despite these results, nanobubble aeration proved to be an efficient method for generating oxygenated and ozonated water treatments. Collectively, this work contributes to better understanding of *C. monteithiana* in Georgia and adds perspective to integrated dollar spot management through evaluation of novel control strategies.

INDEX WORDS: Dollar Spot, *Clarireedia*, Turfgrass, Genetic Diversity, Population Structure, Disease Management, Ultraviolet-C, Nanobubble

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# DEDICATION

To my parents (John and Susan Spratling), grandparents (Willis and Joan Roberson; Jack and Madge Spratling), and wife (Abby Spratling). To my late cousin, Emily Roberson, whose beautiful memory continues to inspire and uplift. To my late mother-in-law, Penny Carson, who left this world a better place.

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## TURFGRASS

In the scope of this work, turfgrass will be referred to as grass species bred and cultivated to tolerate traffic and low mowing heights (<15 centimeters) and that are commonly used in lawns, sports fields, golf courses, athletic fields, and other areas for residential, recreational, and commercial purposes around the world.

### Brief history and significance of turfgrass

The origins of modern day turfgrasses stretch back thousands of years and are associated with the global domestication of grazing animals (Beard, 1998). This domestication spurred changes in natural grassland species that resemble features we see in turfgrasses today (Jacobs et al., 1999). The idea of intentionally managing grasses was likely also conceived from the period of animal domestication. Roberts et al. (1992) suggested that the herding of animals created dense sods that coaxed humans to design ball games. The creation of these ancient ball games and other similar activities called for grass fields that supported participants and facilitated playability. Natural selection probably played the most significant role in grass coverage on these earlier "fields" rather than actual management, but humans likely modified playing surfaces where they could. The earliest reports of more deliberate grass management are documented around the 11$^{th}$ century, when grasses became an integral part of many European gardens (Aldous, 2014). The advent of the game of golf in the 15$^{th}$ century further provoked the

discipline of grass management (Darwin, 1952). Fast forwarding to the early 19th century, grass management was revolutionized with Edwin Budding's invention of the lawn mower (Okafor, 2013). The mower replaced outdated scythes and horse-drawn carriages and served as the cornerstone for development of other grass management equipment. Grass management continued to improve and evolve as sport and recreation became more important to humans.

Today, turfgrass is the most widely used vegetative groundcover in the United States, and managed turfgrass areas comprise approximately 2% of the entire continental land area (Milesi et al., 2005; Phillips et al., 2023). Over 50 million acres of turfgrass span landscapes nationwide, and over 30 million of these acres are irrigated, which establishes turfgrass as the largest irrigated crop in the country (Morris, 2003b). There are approximately 25 to 30 million acres of home lawns across the country (Balogh et al., 2020), along with an estimated 700,000 athletic fields and nearly 14,000 golf courses comprised of maintained turfgrass (Crompton and Nicholls, 2020; Morris, 2003b). The only crops grown in the United States that surpass turfgrass in acreage are soybeans, corn, barley, and wheat (USDA, 2019).

The importance of turfgrass is not only reflected by its vast acreage grown across the country, but also by its significance to the national economy. The turfgrass industry itself contributes a total economic output and value of US$100 billion to the U.S. economy and supports more than 800,000 jobs (Haydu et al., 2006; Shaddox et al., 2022). The game of golf alone contributes an estimated $20 billion dollars to the U.S. economy per year (Stier et al., 2020), making it the largest sector of the turfgrass industry. In Georgia, the turfgrass industry contributes over $7 billion annually to the state economy and generates over 111,000 jobs. There are over 1 million acres of home lawns in the state, and Georgians spend over $2 billion annually in lawn maintenance. The Georgia landscape industry contributes over $3 billion to the state

economy and generates over 10,000 jobs. Additionally, Georgia's golf industry is valued at over $2.5 billion and supports over 45,000 jobs (University of Georgia, 2019; Waltz, 2020b).

**Purposes and benefits of turfgrass**

Turfgrasses are used for many different purposes and, depending on their intended use, are generally classified into three major categories: utility turf, lawn turf, and sports turf. (Turgeon, 2002). Turfgrass management goals for each of these categories revolve around maintaining visual or functional quality (Morris and Shearman, 2008). Visual quality factors include characteristics such as density, color, uniformity, texture, and smoothness. Functional quality factors are more important in sports turfs and may include rigidity, rooting, resiliency, and elasticity (Morris and Shearman, 2008). Of the three turf types, utility turfgrasses require the least amount of maintenance. They are mostly used for soil stabilization purposes and are found in commons areas such as roadsides, airports, or industrial sites. Some popular utility turfgrasses include bahiagrass, buffalograss, and tall fescue. Lawn turfgrasses are primarily used in ornamental settings to add aesthetic value to landscapes, and these grasses can be virtually any species. Lastly, sports turfs are used on golf courses and athletic fields to provide suitable playing surfaces for athletes. These grasses are intensely managed and kept at low (<2.5 centimeters) mowing heights. Some examples of popular sports turfgrasses include bermudagrasses, zoysiagrasses, and bentgrasses (Turgeon, 2002).

While the functional roles of turfgrasses are evident in society, they also provide other various comforts that enhance overall quality of life for humans. For example, turfgrasses often comprise communal greenspaces that supply numerous health benefits. Living in close proximity to these greenspaces has been shown to reduce stress and symptoms of depression by providing a

connection with nature that creates a sense of serenity (Barrett et al., 2014; Beyer et al., 2014; Frumkin, 2001). Additionally, green landscapes have also been shown to enhance cognitive, intellectual, and creative skills in children (Frumkin, 2001; Heerwagen and Orians, 2002). Barrett et al. (2014) found that people with easy access to parks and other greenspaces were more physically active and therefore had a reduced risk of developing chronic diseases. Similarly, Bell et al. (2008) and Liu et al. (2007) found that green neighborhoods were associated with reduced body mass index (BMI) in children due to the promotion of physical activity. Overall, the aesthetic and recreational contributions of turfgrasses to communities can enhance mental well-being, promote active living, and foster social interaction (Beard and Green, 1994; Haskell et al., 2007; Kaplan, 2001).

In addition to societal benefits, the environmental benefits of turfgrasses are also well-documented. Dense turfgrass stands reduce overland waterflow during rainfall events, which diminishes soil displacement and prevents pollutant contamination in bodies of water (Gross et al., 1991; Morton et al., 1988). Their dense, fibrous root systems also make turfgrass species good candidates for vegetative groundcover in land reclamation projects (Stier et al., 2013). Additionally, several studies have shown the effectiveness of turfgrass in reducing atmospheric temperatures through evapotranspiration processes (Wang et al., 2016; Wu et al., 2007), which in turn helps reduce energy input and costs required for indoor cooling in urban environments (Beard and Johns, 1985). Similarly, other studies have shown that turfgrass dissipates radiant heat through evaporative cooling, which reduces heat island effects in cities (Amani-Beni et al., 2018; Jenerette et al., 2011). Other environmental benefits that turfgrasses provide include enhancing groundwater recharge, reducing urban noise and glare, reducing wildfire risk, and creating wildlife habitats (Monteiro, 2017).

**Turfgrass classification and taxonomy**

Turfgrasses are split into two major groups: cool-season and warm-season grasses. The distinction between these two groups is related to differing photosynthetic metabolisms. In cool-season grasses, carbon dioxide is initially fixed into 3-carbon molecules using RuBP carboxylase during the $C_3$ cycle. Consequently, these grasses are commonly referred to as $C_3$ grasses. Warm-season grasses are commonly referred to as $C_4$ grasses, as they initially fix carbon dioxide into 4-carbon molecules via PEP carboxylase in the $C_4$ cycle (Ehleringer and Cerling, 2002). The distinct photosynthetic pathways of these two groups correspond to their respective thermotolerances. The optimal temperature range for $C_3$ grass growth is 15 to 24˚C (59 to 75˚F), whereas the optimal range for $C_4$ grasses is 26 to 35˚C (78 to 95˚F). $C_3$ grasses show bimodal growth patterns, where root and shoot growth is most vigorous in the spring and fall and slows down or stops completely in the summer (Huang and Jiang, 2002). The halt of summer growth is caused by high temperatures that induce high rates of photorespiration, which drastically reduces net photosynthetic activity (Chollet and Ogren, 1975). Oppositely, most of the root and shoot growth in $C_4$ grasses occurs from late spring through early fall and is diminished in winter months (Turgeon, 2002). Photorespiration in $C_4$ grasses is virtually non-existent (Chollet and Ogren, 1975).

In terms of taxonomical classification, all grass species belong to the *Poaceae* family. The three main subfamilies within *Poaceae* that constitute turfgrasses include *Pooideae, Panicoideae*, and *Chloridoideae*. The *Pooideae* subfamily contains all $C_3$ turfgrasses. It is made up of sixteen tribes, but only the Poeae tribe contains major turfgrass genera. These genera include *Festuca* (fescue), *Poa* (bluegrass), *Lolium* (ryegrass), and *Agrostis* (bentgrass). Species

within these genera comprise the most widely grown $C_3$ turfgrasses in the United States (ITIS, 2021; NCBI, 2020; Soreng et al., 2017).

$C_4$ turfgrasses belong to *Panicoideae* or *Chloridoideae* subfamilies. The three tribes within the *Panicoideae* subfamily that contain major turfgrass genera include Paspaleae, Paniceae, and Andropogoneae. The Paspaleae tribe contains *Axonopus* (carpetgrass) and *Paspalum* (bahiagrass and seashore paspalum) genera, the Paniceae tribe contains the *Stenotaphrum* (St. Augustinegrass) genus, and the Andropogoneae tribe contains the *Eremochloa* (centipedegrass) genus. The *Chloridoideae* subfamily comprises six tribes, but only the Cynodonteae and Zoysieae tribes contain major turfgrass genera, including *Cynodon* (bermudagrass) and *Zoysia* (zoysiagrass), respectively. Species within the aforementioned genera represent the most widely grown $C_4$ turfgrasses in the United States (ITIS, 2021; NCBI, 2020; Soreng et al., 2017).

**Turfgrass adaptation in the United States and Georgia**

As their names imply, the viability and effective establishment of cool-season and warm-season turfgrasses are directly linked to climate. Climate is the most important factor in determining the suitability of a turfgrass species for a given region (Ward, 1969). Of course, there are several environmental factors that constitute the climate of a specific region, but the most important factors pertaining to turfgrass adaptation are temperature and precipitation (Hatfield, 2017). Christians et al. (2016a) developed a climatic map for the United States that partitions the country into ten specific zones of turfgrass adaptation, each distinguished by different temperature and precipitation patterns. Based on this map, cool-season grasses are most competitive in cool humid and cool arid zones that span the Pacific Northwest, Midwest, and

Northeast regions of the country. Warm-season grasses grow best in tropical, warm humid, and warm arid zones that span the southernmost portions of the Southeast and Southwest regions of the United States. Transition zones, where neither warm-season nor cool-season grasses are ideally well-adapted, stretch across a central part of the country from Virginia to Arizona (Christians et al., 2016a; Cook and Ervin, 2010). It is important to note that while certain turfgrasses are better adapted to certain climatic zones, they can be grown outside these zones if conditions permit. For example, the climate in the cool humid zone of the Pacific Northwest becomes suitable for zoysiagrass ($C_4$ turfgrass) growth in the summer, but this period only lasts for about two months. Similarly, because of its exceptional shoot density and dark green color, creeping bentgrass ($C_3$ turfgrass) is commonly used on putting greens in the warm humid zone of the Southeast, but it becomes difficult to manage as temperatures rise during the middle parts of the year (Christians et al., 2016a).

In the state of Georgia, five distinct geographic regions are typically recognized: Valley and Ridge, Appalachian Plateau, Blue Ridge, Piedmont, and Coastal Plain (Usery, 2016). The two largest regions, the Piedmont and the Coastal Plain, are adjacent to each other and are delineated by the Fall Line. The Fall Line cuts across the state from Columbus, to Macon, to Augusta. The Coastal Plain is located below the Fall Line and comprises most of the southern half of the state. The Piedmont region sits above the Fall Line and extends across the state up to Carrolton and Toccoa. The Appalachian Plateau and Valley and Ridge regions make up the upper northwest portion of the state, and the Blue Ridge region makes up the upper northeast part of the state (Usery, 2016). In the context of U.S. turfgrass adaptation zones, the Valley and Ridge, Appalachian Plateau, upper Piedmont, and Blue Ridge regions are part of a transitional

zone. The lower portion of the Piedmont and the Coastal Plain are part of the warm humid zone (Christians et al., 2016a).

Georgia's geographic and climatic diversity allows for extensive use of both $C_3$ and $C_4$ turfgrasses. $C_4$ grasses such as bermudagrasses, zoysiagrasses, and centipedegrass are grown statewide. Seashore paspalum is best suited for the Coastal Plain region, while St. Augustinegrass is usually grown in both the Coastal Plain and Piedmont regions (Waltz, 2020a). $C_3$ grasses such as Kentucky bluegrass and fine fescues are generally constrained to the mountainous Blue Ridge and Ridge and Valley regions. Tall fescue is also utilized in these mountainous regions and in the Piedmont. Ryegrasses are grown statewide (Waltz, 2020a).

**Turfgrass management**

Turfgrass management strategies depend on the species of turfgrass being grown and their intended end use. As previously mentioned, turfgrasses used for sports, such as those used on golf courses, require the most maintenance. Golf course turfgrasses are measured by both visual and functional quality, whereas turfgrasses grown in other settings such as home lawns or recreational landscapes are often judged solely on visual appeal. The demand for maintaining aesthetic quality while facilitating playability makes turfgrass management on golf courses particularly challenging. However, much of the evolution and progression in turfgrass science is borne out of meeting this demand. Additionally, the golf sector is the probably the largest and most economically significant branch of the turfgrass industry (Haydu et al., 2008), and is where the most research on cultural management has been conducted. Therefore, the general overview of turfgrass management presented in the following sections will be in the context of golf course turfgrass management.

### *Major turfgrass species of southeastern U.S. golf courses*

Turfgrass management varies for different parts of a golf course but always starts with choosing the proper turfgrass species for a given area. Bermudagrasses, zoysiagrasses, seashore paspalum, and creeping bentgrass are the most widely used golf course turfgrasses in the southeastern U.S. Bermudagrasses are deep-rooted and produce vigorous, dark-green colored turfgrass stands when managed properly. They grow by stolons and rhizomes, which gives these grasses excellent recuperative ability and resistance to certain environmental stresses. However, bermudagrasses generally have poor shade tolerance and become dormant and turn brown in cold weather (McCarty, 2011). Many golf course superintendents overseed ryegrasses into bermudagrass stands just before the onset of dormancy to maintain year-round green color. Bermudagrasses are utilized on golf course tees, fairways, greens, and roughs, and popular cultivars include 'TifEagle', 'Tifway 419', 'TifTuf', 'Tahoma 31', 'MiniVerde', and 'Champion' (Gopinath, 2020; Morris, 2003a).

Zoysiagrasses are becoming popular golf course grasses due to their improved winter hardiness and shade tolerance in comparison to bermudagrass. Zoysiagrasses also have great wear tolerance and salt tolerance and typically display a lighter shade of green than bermudagrass (McCarty, 2011). Zoysiagrasses are slow to establish, which can be advantageous or disadvantageous depending on where they are planted on the golf course. These grasses are often used for fairways, tees, and sometimes greens, and popular zoysiagrass cultivars include 'Zeon', 'Meyer', 'Empire', 'Emerald', 'Zorro', and 'El Toro' (Patton, 2010; Unruh et al., 2022).

Seashore paspalum is not as popular as bermudagrass or zoysiagrass for golf courses in the Southeast but is renowned for its salt tolerance. It has the highest salt tolerance of all $C_4$ turfgrasses, so it is often utilized on seaside golf courses. In comparison to bermudagrass,

seashore paspalum has better shade and cold weather tolerance and displays a shiny, dark-green hue when managed properly (Brosnan and Deputy, 2008). Seashore paspalums are used on golf course greens, fairways, and tees, and popular cultivars include ‘Seastar’, ‘Sea Isle I’, ‘Sea Isle 2000’, ‘SeaDwarf’, and ‘Platinum TE’ (Alabi, 2023; Crawford, 2014).

Finally, creeping bentgrass is by far the most popular $C_3$ grass used on golf courses in the Southeast, but it is only used on putting greens. Being a cool-season grass, creeping bentgrass becomes challenging to manage during hot weather, so managing stands on scales larger than that of a putting green would be impractical. Despite its inability to thrive in hotter climates, creeping bentgrass can make for an exceptional putting surface due to its vigorous, stoloniferous growth habit. Popular bentgrass varieties include ‘Pencross 2.0’, ‘A1/A4’, ‘007’, and ‘L-93’, and they produce colors ranging from dark blue-green to greenish-yellow (Bigelow and Tudor, 2011; Morris, 2003a; USGA Green Section Staff, 2024). Creeping bentgrass is more shade tolerant than bermudagrass, has similar fertility requirements, but is generally less tolerant to wear (Beard, 2002).

***Fertilization***

Proper fertilization is essential to any golf course turfgrass management program to sustain desirable color, density, recuperation, vigor, and resistance to pests. Important turfgrass nutrients that are primarily obtained from fertilization include nitrogen (N), phosphorus (P), and potassium (K), known as the macronutrients. Of these three nutrients, nitrogen is the most important for turfgrasses and is required in the greatest amount. Nitrogen is a part of amino acids, chlorophyll, hormones, nucleic acids, and nucleotides (Marschner, 2011) and usually comprises 2 to 6% of dry turfgrass leaf tissue (Mills and Jones Jr., 1996). It affects several turfgrass traits including color, shoot density, recuperative ability, and root, rhizome, and stolon

growth (Carrow et al., 2002). The second most important of these three nutrients is potassium. Potassium is involved in maintaining cellular turgor pressure and in opening and closing of stomata. It is also involved in enzyme activation, protein synthesis, and translocation of assimilates (Frank and Guertal, 2013). Lastly, phosphorus is required in the least amount when compared to nitrogen and potassium but is still crucial for turfgrass growth and development. Phosphorus is involved in energy transfer via adenosine triphosphate (ATP) in numerous metabolic processes and is also a constituent of phospholipids, nucleic acids, and several coenzymes. It influences seedling development, root growth, and maturation of turfgrass plants (Frank and Guertal, 2013).

Other nutrients important for turfgrass plants include iron, zinc, copper, manganese, boron, chloride, molybdenum, and nickel (the micronutrients). Southeastern soils usually contain a sufficient amount of these micronutrients to support turfgrass growth, and most premium turfgrass fertilizers include these nutrients in mixes. Therefore, micronutrient deficiencies are rather rare in golf course turfgrasses. However, of the deficiencies that do occur, iron deficiency is the most common (McCarty, 2011). This deficiency is most likely to occur on alkaline soils just after spring green-up. Iron deficiency symptoms in a turfgrass stand involve interveinal chlorosis in younger leaves, and older leaves display chlorosis if iron is not applied promptly (Wehner, 1992). Superintendents apply iron in liquid form as needed when deficiency symptoms start to appear.

In terms of timing fertilizer applications, particularly N applications, the turfgrass plant must be actively growing to absorb any nutrient. As previously mentioned, turfgrass root and shoot activity is temperature-dependent, with cool-season grasses being most active between 15 to 24˚C (59 to 75˚F) and warm-season grasses being most active between 26 to 35˚C (78 to 95˚

F) (Huang and Jiang, 2002). Therefore, most N applications occur in the fall and spring for cool-season grasses and in the spring and summer for warm-season grasses. Moreover, different turfgrass species require different amounts of N to maintain optimal color, density, and vigor. Bermudagrass greens typically require 0.5 pounds of N per 1,000 square feet every 7 to 14 days during the growing season. Similar rates of N are applied to bermudagrass fairways and tees less frequently (roughly monthly basis) during the growing season (McCarty, 2011). Zoysiagrass fairways and tees are often fertilized on a similar schedule to bermudagrass, but they typically require less N per application (0.2 to 0.4 pounds of N per 1,000 square feet) (Beard, 2002). Seashore paspalum fairways and tees respond well to 5 to 8 pounds of N per 1,000 square feet per year, and seashore paspalum greens prosper with 3 to 6 pounds of N per 1,000 square feet annually (Brosnan and Deputy, 2008). Lastly, creeping bentgrass greens typically require 3 to 6 pounds of N per 1,000 square feet annually (Beard, 2002).

***Irrigation***

Irrigation requirements for turfgrass stands are affected by environmental conditions, turfgrass species, and soil type or condition (Christians et al., 2016b). Environmental conditions have a large effect on evapotranspiration, which is the combined water loss from the soil surface (evaporation) and from the plant (transpiration). The rate of evapotranspiration in a turfgrass stand is often used to calculate irrigation demands, and different turfgrass species have different evapotranspiration rates. $C_3$ species typically have higher rates than $C_4$ species due to their inefficiencies in photosynthesis. Both $C_3$ and $C_4$ grasses close stomata during periods of heat stress to prevent water loss, but $C_3$ grasses do not tolerate these periods as well due to the limited entry of carbon dioxide into cells (Ehleringer and Pearcy, 1983; Taylor et al., 2014). During photosynthesis, when carbon dioxide levels are low, $C_3$ grasses start fixing oxygen into

phosphoglycolate (photorespiration) instead of fixing carbon dioxide into sugars (Osmond et al., 1982). To prevent this wasteful process, $C_4$ grasses instead use a two-stage photosynthetic method that consistently maintains high carbon dioxide levels in cells (Ehleringer and Pearcy, 1983; Hatch and Osmond, 1976).

Determining the exact timing and quantity of water needed for golf course irrigation can be challenging due to the variety of terrains, environmental conditions, and grass types inherent to golf courses. However, a very general rule of thumb for golf course irrigation is that a high-quality turfgrass stand may require up to 4.5 centimeters of water per week including rainfall (Murphy, 2002). To gauge irrigation requirements more precisely, golf course irrigation teams rely on devices such as tensiometers, evaporatory pans, atmometers, and firmness meters to determine soil water status and evapotranspiration rates (Christians et al., 2016b). In terms of scheduling, irrigation is typically most effective during early morning hours due to the cooler temperatures that reduce evaporative losses. Watering in the early morning also allows the turfgrass canopy to dry out as the sun rises, which prevents extended periods of leaf wetness that can promote disease. (Christians et al., 2016b).

***Mowing***

Mowing is the removal of shoot tissue from a turfgrass plant and is arguably the most crucial cultural practice in managing turfgrass stands. It is important to note that photosynthesis occurs in shoot tissues, so removing them has a strong effect on plant physiology and development (Howieson and Christians, 2008; Law et al., 2016). In other words, mowing always stresses a turfgrass plant to some extent. Improper mowing can result in excessive stress, which leads to poor quality by weakening root systems and diminishing color and density (Juska and Hanson, 1961). Mowing requirements differ based on turfgrass species and cultivar, but adhering

to the one-third rule of mowing is generally beneficial for maintaining any turfgrass stand. This rule states that mowing should be performed when turfgrass plant height is one-third higher than the desired height. Removing more than one-third of the leaf blade is an excessive loss of leaf area for a turfgrass plant and can lead to reductions in photosynthetic efficiency and recuperative ability (Reicher et al., 2006). Given this rule, shorter-cut grasses need to be mowed more frequently than higher-cut grasses.

Mowing regimens differ for each component of a golf course hole. In the southeastern U.S., golf course tees consisting of zoysiagrass, bermudagrass, or seashore paspalum are typically mowed two to five times a week and are kept at heights ranging from 0.795 to 1.588 centimeters. Bermudagrass, zoysiagrass, and seashore paspalum fairways are also typically mowed two to five times per week and are kept at heights ranging from 1.113 to 2.223 centimeters. Golf course roughs can vary widely in height, depending on how challenging the superintendent wants to make the course. Most roughs in the southeastern U.S. are mowed one to two times per week and are kept at 2.540 to 7.620 centimeters in height. Lastly, greens consisting of bermudagrass, seashore paspalum, or bentgrass are usually mowed on a daily basis at 0.318 to 0.635 centimeters (McCarty, 2011). An important factor to take into consideration when mowing greens is the direction that grass blades are growing and leaning, known as the grain. If a green is frequently mowed in one direction, the grain becomes more pronounced in that direction (Beard, 2002). This ultimately affects how true and fast a golf ball rolls on a green. To avoid issues with grain, superintendents adjust mowing patterns to influence the direction of grass growth.

***Cultivation***

There are many cultivation practices in turfgrass management that differ from cultivation in other branches of agriculture. For example, in a traditional row crop setting, a grower may till

or plow a field before a growing season to improve soil tilth or incorporate soil amendments. However, this type of traditional cultivation is obviously not applicable in turfgrass settings, as it would destroy a turfgrass stand. Instead, turfgrass practitioners use alternative forms of cultivation that are much less destructive and enable year-round playability and quality. Such practices include coring, slicing, spiking, and vertical mowing. Of these, coring, also known as aerification or aeration, is likely the most important and widely used cultivation practice in turfgrass management. Coring is often done to relieve soil compaction, which is mostly caused by vehicular or foot traffic on golf courses. Soil compaction is the compression of soil constituents into dense masses that leads to inadequate soil aeration, decreased nutrient availability, and poor drainage (Turgeon, 2002). These conditions diminish the overall quality of a turfgrass stand by weakening root systems. Coring relieves compaction by extracting soil cores from a stand with hollow tines or spoons, which allows for improved gas exchange, response to fertilizers, water infiltration capacity, and overall growth (Murphy and Rieke, 1987). Because coring needs to coincide with actively growing turfgrass roots and shoots, it is usually done in late spring or summer for warm-season grasses and in spring and fall for cool-season grasses. A practice that is often done subsequently to coring is topdressing. Topdressing is the application of a thin layer of soil or sand to a turfgrass stand. Topdressing promotes growth to fill in holes after coring, as well as smooths playing surfaces, improves recuperative ability, and reduces thatch (Davis, 1978).

As previously alluded to, a few other forms of cultivation used in turfgrass management include slicing, spiking, and vertical mowing. In slicing, V-shaped knives on mounted discs penetrate the soil to a depth of about 7.620 to 10.160 centimeters. Spiking is similar to slicing except that penetration depth into the soil is limited to only about 2.540 centimeters (Turgeon,

2002). These practices serve similar purposes as coring but are less disruptive, as they do not remove turfgrass or soil material. Since they are less disruptive, they can be performed more often than coring. Furthermore, vertical mowing uses vertical knives that rotate rapidly on a horizonal shaft and penetrate the soil at different depths. Shallower penetration can break up leaves and stolons to reduce graininess of greens, while deeper penetration removes thatch and can relieve some soil compaction (Murray and Juska, 1977).

## Important biotic diseases of southeastern U.S. turfgrasses

Couch (1995) defines plant disease as "an aberrant form of metabolism, incited by components of biological or physical environments, manifested by altered physiology of one or more cells." Although this definition implies that both biotic and abiotic agents cause disease, the following sections will only cover major diseases of southeastern turfgrasses caused by biotic agents. Biotic agents that cause most plant diseases include fungi, nematodes, viruses, and bacteria. Among these, fungi cause the vast majority of turfgrass diseases, and over 150 fungal species have been recognized as turfgrass pathogens (Couch, 1995). Consequently, more than $80 million is spent annually on turfgrass fungicides in the United States, which accounts for over 20% of the national fungicide market (Nelson and Boehm, 2002; Vargas Jr., 2005). Across U.S. golf courses alone, estimated spending on fungicides exceeds $40 million annually (Nelson et al., 2020). Moreover, in comparison to other crops grown across the country, more fungicides are used in turfgrass than in any other commodity (Vargas Jr., 2005).

### *Foliar diseases*

The major foliar diseases of southeastern turfgrasses include dollar spot, *Bipolaris* leaf spot, anthracnose, gray leaf spot, brown patch, and rusts. Dollar spot, caused by *Clarireedia* spp.,

is a persistent and widespread disease that occurs on all $C_4$ and $C_3$ turfgrass species. Creeping bentgrass, bermudagrasses, zoysiagrasses, and seashore paspalum, which comprise the majority of turfgrass species used on golf courses in the Southeast, are all susceptible to the disease (Allen et al., 2012). Dollar spot is particularly damaging in intensely managed turfgrasses, such as golf course putting greens. It causes small, blighted, sunken patches to form in turfgrass stands, which detract from overall aesthetic value and playability (Couch, 1995). A more comprehensive review of dollar spot is described in the latter half of this chapter.

*Bipolaris* leaf spot, caused by *Bipolaris cynodontis*, is a chronic disease of bermudagrass, especially bermudagrass putting greens. The fungus attacks older leaves first, forming water-soaked, red to purple lesions. The pathogen can also attack crowns of turfgrass plants, leading to rot. If left untreated, leaf spot can lead to melting out, which often manifests as thinning or blighting in the form of irregularly shaped patches (Brecht et al., 2007). Melting out symptoms are often more severe in humid, hot conditions of late spring and summer. Spread of *Bipolaris cynodontis* throughout a turfgrass stand is facilitated through the production and dissemination of brown, multicellular, ellipsoidal conidia (Manamgoda et al., 2014).

Anthracnose can be a foliar disease of turfgrass or a basal rot of lower stems (crown disease) (Settle et al., 2006). It is caused by *Colletotrichum cereale* and is a major disease of creeping bentgrass, especially during periods of summer stress. Disease symptoms manifest as irregularly shaped patches of tan to brown turfgrass, ranging from a few centimeters to several meters in size (Latin, 2015). In diagnosis of anthracnose, the presence of acervuli and dark, black setae on the leaf surface are the most recognizable signs of the disease. Additionally, conidia that are hyaline, crescent-shaped, and single-celled may be observed under a microscope (Khan and Hsiang, 2003; Settle et al., 2006).

Gray leaf spot, caused by *Pyricularia grisea,* is a major disease of perennial ryegrass and St. Augustinegrass. Perennial ryegrass is a popular species used for overseeding $C_4$ grasses during periods of dormancy, and St. Augustinegrass is a popular lawn grass in the Southeast. Symptoms of gray leaf spot vary for each species. In perennial ryegrass, the disease can cause severe damage to seedlings in late summer and fall, and symptoms in established perennial ryegrass stands include foliar blighting and dieback in the form of irregularly shaped patches that are yellow to orange in color. On individual perennial ryegrass leaves, gray leaf spot lesions first form along the margins of leaf blades and are gray with brown borders (Tani and Beard, 1997). In St. Augustinegrass, lesions are much larger and more blue-gray in color, and they may have a depressed center with a yellow border. Gray leaf spot dieback in St. Augustinegrass does not manifest in distinct patches, but widespread foliar thinning occurs if the disease is left untreated for extended periods of time. Symptoms of gray leaf spot in a St. Augustinegrass stand are more severe under hot, humid conditions (Tani and Beard, 1997). Microscopically, the *Pyricularia grisea* pathogen produces hyaline, pyriform, two- to three-celled conidia (Martinez-Espinoza et al., 2022).

Brown patch occurs in cool-season turfgrasses and is caused by *Rhizoctonia solani* (strains AG-1 1A and AG2-2 IIIB). All cool-season turfgrass species are susceptible to *R. solani* infection. Brown patch symptoms are most severe in the summertime during hot, humid weather. Disease symptoms manifest as brown or orange circular patches ranging from a few centimeters to several meters in diameter, and a black or dark gray “smoke ring” may surround the patches in closely mowed grasses when humidity is high. Individual leaves may display tan lesions with purplish-brown borders, but lesions are often not present in closely mowed turfgrasses. Rotting at the base of the leaf blade can also occur, making removal of the entire leaf blade from the

sheath very easy (Anderson, 1982; Martinez-Espinoza et al., 2009). *R. solani* produces hyphae that are characterized by regular septations, right-angle branching, and constrictions at branching origins; the pathogen does not produce any spores (Tredway and Burpee, 2001).

Finally, rust diseases commonly occur in the southeastern U.S. on ryegrasses and zoysiagrasses. They are caused by several *Puccinia* spp. and are most severe on slow growing grasses grown in moist, low-light areas (Martinez-Espinoza et al., 2009). Ryegrasses are most susceptible to rusts in the spring when nitrogen fertility is low, and zoysiagrasses are susceptible in the spring or fall when growth rate of the grass slows (Duble, 2001). Yellow to orange foliar lesions and orange-colored pustules form on individual leaves, and turfgrass stands cast a dull, yellow-green color as disease progresses. After multiple cycles of rust infection, turfgrasses may thin or die out due to the reduction of photosynthesis caused by lesion and pustule formation (Obasa and Kennelly, 2010).

***Crown and root diseases***

The major crown disease of southeastern turfgrasses is large patch, and the major root diseases include take-all root rot, spring dead spot, and pythium root rot. Like brown patch, large patch is also caused by *Rhizoctonia solani* (strain AG 2-2 LP), but it only occurs in warm-season grasses. All warm-season turfgrass species are susceptible to *R. solani* infection, but zoysiagrass is most susceptible. The large patch pathogen is most active during warm, humid conditions, and it infects leaf sheaths as it spreads radially in upper soil and thatch layers (Smiley et al., 1992). Large patch symptoms often occur in the spring and fall and appear as thinned turf in circular patches. Patches can range from 1 to 8 meters in diameter and can coalesce to form larger areas of blighted turf. A yellow-orange ring may be present at the perimeter of a patch where infection progresses. Reddish-brown or black lesions form on individual leaf sheaths, and leaf dieback

generally starts from the leaf tip (Green et al., 1994; Martinez-Espinoza et al., 2009). Since large patch infection begins in leaf sheaths near the base of a turfgrass plant, it is considered a crown disease rather than a true foliar disease.

*Gaeumannomyces graminis* var. *graminis* is the causal agent of take-all root rot in warm-season grasses. The pathogen is ectotrophic, meaning it infects turfgrass roots and stolons externally via thick runner hyphae. Runner hyphae are readily seen under a microscope on stolons and roots, as well as popcorn-shaped hyphopodia that serve as attachment points for the fungus (Elliott et al., 1993). Black lesions form on infected roots and stolons, and roots become stunted as infection progresses. Individual leaves are initially chlorotic and eventually turn brown and wilt. Conditions that favor take-all root rot include high rainfall and warmer temperatures of the summer and late fall. Additionally, any biotic or abiotic stressors affecting a turfgrass stand increases susceptibly to the disease. St. Augustinegrass and bermudagrass are particularly susceptible to take-all root rot (Elliott et al., 1993). Disease symptoms in St. Augustinegrass initially appear as irregular chlorotic patches that range from a few centimeters to several meters in diameter. Patches thin as the disease progresses, and infected areas eventually become devoid of turfgrass plants (Elliott and Harmon, 2014). Symptoms in bermudagrass are similar, with initial symptoms consisting of yellowing leaves and darkening roots. As infection progresses, thinning occurs in the form of irregularly shaped patches, and infected areas continue to decline until they are left barren (Smiley et al., 1992).

Spring dead spot, caused by *Ophiosphaerella* spp., is one of the most damaging diseases of bermudagrass. Despite its name, spring dead spot infection begins during cooler conditions of the fall. Throughout the fall, the pathogen colonizes rhizomes, stolons, and roots of hosts using dark-brown ectotrophic runner hyphae, similar to *Gaeumannomyces graminis* var. *graminis*

(Vincelli, 2021). This initial infection can lead to necrosis prior to winter dormancy, but the most severe symptoms are often observed during the following year's spring green-up period. Spring dead spot infection progresses through the fall and winter, and infected plant tissues do not survive winter dormancy. As a bermudagrass stand transitions out of dormancy into active spring growth, spring dead spot symptoms manifest as circular or semicircular patches of dead grass ranging up to 0.3 meters or more in diameter. Uninfected plants that are adjacent to these patches will regrow normally, causing sharp divides to form between healthy and diseased areas (Wadsworth and Young Jr., 1960).

Pythium root rot (PRR), caused by multiple *Pythium* species (oomycetes), is a major disease of creeping bentgrass in the southeastern U.S. It can also be problematic in some warm-season species. Since many *Pythium* species can cause root rot, infection can occur at any given time during a growing season. Additionally, multiple *Pythium* species can simultaneously infect or be present on turfgrass roots, which can make diagnosis of PRR tricky (Hodges and Campbell, 1994). Usually, thick, round, double-walled oospores and coenocytic hyphae can be seen under a microscope in symptomatic root tissues and are diagnostic for PRR. Furthermore, *Pythium* species that cause PRR are often categorized into cool- or warm-season groups based on temperature ranges that coincide with pathogen activity. Cool-season *Pythium* infection usually occurs at 12 to 22˚C (55 to 70˚F), while warm-season species infection occurs at temperatures above 29˚C (85˚F) (Downer and Harivandi, 2016). Moreover, *Pythium* infections are most severe in areas with high soil moisture or poor soil drainage. Initial symptoms of PRR in a creeping bentgrass stand include thinning and slight necrosis of small areas of turfgrass. These areas coalesce and die out if left untreated. Infected roots often have tan lesions and are brittle and stunted (Hampy et al., 2021).

### *Fairy ring*

The pathogens described above infect root, crown, or foliar turfgrass tissues. However, there is a notable disease of turfgrass called fairy ring that does not directly parasitize any part of a turfgrass plant. Fungi that cause fairy ring dwell in soil or thatch and feed on organic matter. The feeding or activity of subsurface fungal colonies and the nature of their mycelium can create hydrophobic soil conditions that indirectly damage turfgrass areas. Additionally, some fairy ring fungi also produce and release organic acids that are harmful to turfgrass tissues (Corwin et al., 2007). Fairy ring symptoms in turfgrass stands typically manifest as rings or arcs that can range in size from 1 to 5 meters in diameter, and most fairy rings produce mushrooms or puffballs that appear along the periphery of these rings or arcs after heavy rains (Couch, 1995). More than 40 species of basidiomycete fungi have been attributed to causing fairy ring, and because these fungi do not directly infect turfgrass tissues, all $C_3$ and $C_4$ turfgrass species are susceptible to the disease. However, it should be noted that not all fairy rings are damaging to turfgrass stands. Some fairy rings actually stimulate turfgrass growth through the breakdown of organic matter that releases nitrates, and others do not afflict or influence turfgrass growth at all (Shantz and Piemeisel, 1917).

## Turfgrass disease management

The most important factor in developing any turfgrass disease management regimen is accurate diagnosis. Turfgrass disease diagnosis can be challenging due to the dynamic nature of turfgrass environments and pathosystems. Additionally, many ailments of turfgrass, whether abiotic or biotic, result in similar symptom expression. Therefore, several factors must be taken into consideration in order to properly diagnose turfgrass issues, some of which include site

history, cultivar or species of turfgrass being grown, season, climate, symptomology, signs, soil conditions, and prior management practices (Couch, 1995). Given the complexities these variables introduce, disease diagnosis is best conducted on a case-by-case basis using a comprehensive, systematic approach. Once a disease of turfgrass is properly diagnosed, disease management involves modifying or eliminating one of the components of the disease triangle, which is an illustrative model that depicts the interactions between three factors that cause disease. These three factors include a susceptible host, a conducive environment, and a virulent pathogen (Agrios, 2005). If all three of these components are present and align favorably, disease will occur, but if one of them is missing or unfavorably aligned, disease cannot occur or is less severe.

***Cultural disease management***

The goal of cultural disease control in turfgrass systems is to create an environment that promotes healthy turfgrass growth to prevent or better tolerate disease (Vargas Jr., 2005). In other words, implementing mowing, irrigation, fertility, and cultivation practices that enhance the vigor a turfgrass stand will naturally help combat disease. Of course, proper implementation of these practices depends on the species or cultivar of turfgrass being grown and the specific environment in which they are grown. It is important to note, however, that in certain situations, cultural practices that favor healthy turfgrass stands may also inadvertently favor disease development or other turfgrass problems. For example, supplemental N fertilization often prevents diseases such as dollar spot and anthracnose but may also promote the development of brown patch, gray leaf spot, and *Pythium* blight (Vargas Jr., 2005). Coring relieves compaction but could provide openings for weed germination (Powell Jr., 2000). Topdressing reduces thatch levels but using unsuitable topdressing mixes could lead to the formation soil layers that act as

barriers to water infiltration (Waddington, 1992). Optimal mowing heights promote good root and shoot growth and recuperation, but mowing with dull blades could create large wounds and more surface area for fungal pathogens to invade (Thurn et al., 1994). Proper irrigation is a crucial component of maintaining a healthy turfgrass stand, but too little can lead to drought stress that makes plants more susceptible to disease, and too much promotes the development and spread of several fungal pathogens (Couch, 1995). Overall, cultural control practices are a vital part of any integrated disease management program, but there is no universal guide to utilizing them in every unique scenario. Turfgrass managers must continually adapt to changing environmental conditions and constantly monitor turfgrass stands in order to implement timely cultural practices that are most effective in promoting plant health and disease abatement.

***Chemical disease management***

As previously mentioned, the majority of turfgrass pathogens are fungi, so fungicides play a crucial role in disease control programs. Fungicides are generally classified as site-specific (single-site) or multi-site, with most modern-day fungicides being site-specific. Site-specific fungicides interfere with specific biochemical reactions of sensitive fungi, and they usually work inside the plant (systemic or penetrative) to alleviate existing infections or to prevent pathogen spread. Because site-specific fungicides only target one specific biochemical process in fungi, they are more prone to resistance development than multi-site fungicides (Cohen and Levy, 1990; Vincelli and Munshaw, 2014). Contrary to site-specific fungicides, multi-site fungicides disrupt numerous metabolic processes within a fungus by targeting multiple sites, and they are not absorbed into plants (contact). Multi-sites have no curative action against existing infections, so they are used to prevent the spread of pathogens to healthy plants. Because these fungicides target multiple sites, fungal pathogens are less likely to develop resistance to them. Hence, they

are often used in tandem with site-specific fungicides to help prevent or delay resistance. (Cohen and Levy, 1990; Vincelli and Munshaw, 2014). Furthermore, fungicides are also grouped by their mode of action (MOA), which describes the specific biochemical process they disrupt within a fungus. There are thirteen major fungicide MOAs, and all but four are used in turfgrass settings (FRAC, https://www.frac.info/home). One of the reasons fungicides are classified by MOA is to encourage pesticide applicators to rotate MOAs. This rotation helps prevent the development of fungicide resistance by reducing the selection pressure associated with repetitive applications of a single MOA (Young and Patton, 2010).

Certain fungicides are effective against certain classes of fungi, and the three primary classes that constitute turfgrass pathogens include oomycetes, ascomycetes, and basidiomycetes. Oomycetes are actually not true fungi and are considered fungal-like. Oomycetes do not have ergosterol in cell membranes and usually do not have hyphal septations (Rossman and Palm, 2006). They reproduce sexually via oospores, and the major oomycete pathogens of turfgrass belong to the *Pythium* genus. Basidiomycetes are true fungi and sexually reproduce via basidiospores (Rossman and Palm, 2006). Their cell walls contain chitin, their cell membranes contain ergosterol, and they often have regularly septate hyphae. Major basidiomycete pathogens of turfgrass include *Rhizoctonia* spp. and fungi associated with fairy rings and rusts. Ascomycetes are also true fungi and reproduce sexually via ascospores (Rossman and Palm, 2006). Like basidiomycetes, they also have cell walls containing chitin, cell membranes containing ergosterol, and regularly septate hyphae. Ascomycetes make up the majority of fungal turfgrass pathogens and are causal agents of diseases such as dollar spot, anthracnose, gray leaf spot, *Bipolaris* leaf spot, and several root diseases. In turfgrass settings, the dithiocarbamate, nitrile, benzimidazole, dicarboximide, demethylation inhibitor (DMI), succinate dehydrogenase

inhibitor (SDHI), strobilurin, and aromatic hydrocarbon fungicide families are used against many basidiomycete and ascomycete pathogens (Latin, 2011). Aromatic hydrocarbons and strobilurins can also suppress oomycetes. Phenylamides, carbamates, phosphonates, and cyanoimidazoles are specifically used for oomycete control (Latin, 2011).

## DOLLAR SPOT OF TURFGRASSES

### Dollar spot discovery and taxonomy

The first report of dollar spot in turfgrass occurred in the United States in 1927 and was documented by a USDA pathologist named John Monteith. Monteith's record was more of a question rather than an official disease report, as it was titled "Can you identify brown patch?" (Monteith, 1927). Monteith and colleagues first referred to the disease as 'smaller brown patch', but eventually moved away from this designation to avoid confusion with a turfgrass disease caused by *Rhizoctonia solani* that caused larger brown patches (Monteith and Dahl, 1932). They later assigned the name 'dollar spot' to the disease, as the small brown patches it produced in turfgrass stands were approximately the size of a silver dollar. Monteith initially considered the casual organism to be a *Rhizoctonia* species, as aerial mycelia and coloration like that of *Rhizoctonia* were observed in culture. Nevertheless, no causal organism was attributed to the disease until 1935, when F.T. Bennet of the University of Durham proposed *Rhizoctonia monteithianum*. However, this designation was never widely accepted. Two years later, based on further observations and isolations, Bennet officially deemed *Sclerotinia homoeocarpa*, a fungal ascomycete, as the causal agent of dollar spot (Bennett, 1937).

While ascribing *S. homoeocarpa* to dollar spot, Bennet described three different strains. The 'perfect strain' developed apothecia, ascospores, conidia, and sclerotial structures. The 'ascigerous strain' produced apothecia, ascospores, microconidia, and sclerotial structures. The 'non-sporing strain' only produced white mycelia and none of the other aforementioned structures. Bennet readily placed the fungus into phylum Ascomycota, subphylum Pezizomycotina, family Helotiaceae, and subfamily Helotiae. However, he acknowledged issues in further classifying the pathogen when he mentioned, "some difficulty arises as to its genus" (Bennett, 1937). *Sclerotinia* fungi develop sclerotia, which are survival structures consisting of a mass of hyphal threads that can germinate to produce other reproductive or vegetative structures. Bennet only observed what he believed to be sclerotia-like structures rather than true sclerotia. This was not an issue until 1945, when the classification of the genus *Sclerotinia* was revised to include species that produced apothecia only from genuine sclerotium. *S. homoeocarpa* did not fulfill this condition and was thus omitted from the genus (Whetzel, 1945). What Bennet initially described as sclerotia-like structures were likely just the early stages of stroma formation in *S. homoeocarpa* cultures. (Baldwin and Newell, 1992; Jackson, 1973; Novak and Kohn, 1991).

Efforts to accurately reclassify the dollar spot pathogen would carry out over the next 50 years, but this task proved challenging because the fungus does not produce many morphologically distinct features that are useful in fungal systematics (i.e. sexual or asexual reproductive structures). Bennet first described the fungus producing spores and fertile apothecial fruiting bodies, but only two other reports corroborated these observations (Baldwin and Newell, 1992; Jackson, 1973). Additionally, these accounts of reproductive structure formation had only occurred for strains collected in the United Kingdom and never for strains examined in the United States. During this era, some suggested the fungus belonged to the genus

*Rutstroemia* (Jackson, 1973; Whetzel, 1946), and others believed that more than one organism was responsible for causing the disease (Baldwin and Newell, 1992; Kohn, 1979). However, there were no standard molecular classification tools available at the time that could definitively settle the issue, so the classification of the dollar spot pathogen remained indefinite well into the 1990s.

The 1990s and early 2000s brought a new wave of researchers armed with advanced molecular technologies that could help resolve dollar spot pathogen classification. Unfortunately, most of the results published during this era were inconsistent. Novak and Kohn (1991) suggested the fungus be reclassified into the genus *Poculum* within the *Rutstroemiaceae* family based on electrophoretic analysis of stromatal proteins. A phylogenetic analysis using internal transcribed spacer (ITS) sequences performed by Carbone and Kohn (1993) showed *S. homoeocarpa* clustering with fungi within the *Rutstroemia* genus. Other phylogenetic studies using ITS sequences showed *S. homoeocarpa* isolates grouping with fungi in both *Poculum* and *Rutstroemia* genera (Holst-Jensen et al., 1997; Powell, 1998). Furthermore, several researchers at the time still suggested that more than one species caused dollar spot (Liberti et al., 2012; Taylor, 2010; Viji et al., 2004). Progress was made during this era, but legitimate reclassification remained unsolved due to contradictory reports.

Finally, in a phylogenetic study conducted in 2018 using three DNA markers (ITS region, calmodulin (*CaM*), and DNA replication licensing factor Mcm7 (*Mcm7*)), Salgado-Salazar et al. (2018) verified that the dollar spot pathogen(s) did not belong to any known fungal genus and that multiple species were responsible for causing the disease. They created a new genus for dollar spot pathogens within the *Rutstroemiaceae* family. The genus was named *Clarireedia*, and it contained four new species: *C. homoeocarpa*, *C. benettii*, *C. jacksonii*, and *C. monteithiana*. *C.*

*benettii* and *C. homoeocarpa* are mostly restricted to the United Kingdom, and they infect $C_3$ grasses. *C. jacksonii* and *C. monteithiana* are globally distributed, with *C. jacksonii* primarily infecting $C_3$ grasses and *C. monteithiana* primarily infecting $C_4$ grasses. (Salgado-Salazar et al., 2018). In addition to the four species described by Salgado-Salazar et al. (2018), Hu et al. (2019) later discovered two more species, *C. paspali* and *C.* aff. *paspali*, which were isolated from seashore paspalum ($C_4$ grass) in China. A few years after that, Zhang et al. (2022) identified yet another species, *C. hainanense*, also isolated from seashore paspalum in China.

***Host specificity***

In their study, Salgado-Salazar et al. (2018) asserted that *C. jacksonii* and *C. monteithiana*, the two most widely distributed *Clarireedia* species, exhibit host specificity, where *C. jacksonii* infects $C_3$ hosts and *C. monteithiana* infects $C_4$ hosts. However, a few studies have shown that both of these species can infect both host types. While assessing genetic diversity of *Clarireedia*, Liberti et al. (2012) found that their collected isolates grouped by two distinct types, a Floridian biotype ('F-type') and a Common biotype ('C-type'). The F-type isolates formed stratified stroma in culture and produced pigments under intense light incubation that the C-type did not. In detached leaf assays, the F-type caused prominent leaf blade yellowing as opposed to water-soaked lesion formation caused by the C-type. The group also found DNA sequence variation in the rDNA small subunit (SSU) region between the two biotypes—the F-type frequently contained an SSU group 1 intron that was never observed in C-type isolates. Considering the morphological, pathological, and genetic differences between F- and C-type isolates, the researchers suggested that they could constitute different species. In terms of host specificity, the group reported that both putative pathogen species were collected from both $C_3$ and $C_4$ turfgrasses (Liberti et al., 2012). Furthermore, through pathogenicity tests

subjecting $C_3$ and $C_4$ grasses to *C. monteithiana* and *C. jacksonii* isolates, Aynardi et al. (2019) found that both species were able to incite disease on both grasses. They also found that *C. jacksonii* was more virulent than *C. monteithiana* (Aynardi et al., 2019). Sapkota et al. (2020) also observed through cross-inoculation experiments that both *C. jacksonii* and *C. monteithiana* were able to infect $C_3$ and $C_4$ hosts. Moreover, in their study describing a new dollar spot pathogen species, Hu et al. (2019) mentioned that *C. jacksonii* isolates in their collection were recovered from both $C_3$ and $C_4$ hosts in China, but *C. monteithiana* isolates they possessed only came from $C_4$ hosts. This report indicates the presence of natural *C. jacksonii* infection occurring in $C_3$ and $C_4$ hosts (Hu et al., 2019). These studies show that host adaptation is likely present in *C. jacksonii* and *C. monteithiana*, which could have significant implications in dollar spot management strategies.

**Distribution and economic impact of dollar spot**

Dollar spot is a prevalent and persistent disease that occurs on all $C_3$ and $C_4$ turfgrasses throughout the world. It is widespread across North America, Central America, Australia, Japan, New Zealand, continental Europe, and the United Kingdom (Couch, 1995). It is the most commonly occurring turfgrass disease in North America (Vargas Jr., 2005). In the United States, the disease is less problematic in the Pacific Northwest and arid regions of the West, but is prevalent elsewhere, especially in temperate or hot humid regions (Vargas Jr., 2005). Dollar spot occurs in turfgrasses grown in a variety of different settings including golf course greens, tees and fairways, athletic fields, home lawns, community landscapes, recreational areas, and more.

*Clarireedia* is capable of causing disease on more than 40 different hosts, most of them belonging to the grass family Poaceae (Walsh et al., 1999). The most important hosts include

major turfgrass species. Significant $C_4$ turfgrass hosts include bermudagrasses, zoysiagrasses, seashore paspalum, St. Augustinegrass, centipedegrass, and bahiagrass. Zoysiagrasses, hybrid bermudagrasses, and seashore paspalum are particularly susceptible to *Clarireedia* infection (Allen et al., 2012), and these three grasses comprise most athletic fields and golf courses in the southeastern U.S. Important $C_3$ hosts include bluegrasses, fescues, ryegrasses, and creeping bentgrass. Perennial ryegrass and tall fescue are generally less susceptible to dollar spot, while creeping bentgrass and annual ryegrass are quite vulnerable (Allen et al., 2012). Creeping bentgrass is likely the most significant $C_3$ turfgrass species affected by dollar spot, as it is a popular choice for golf course greens and fairways throughout cool and temperate regions of the United States. There are cultivars of creeping bentgrass that are less susceptible to dollar spot than others (Sapkota et al., 2022), but the disease is still burdensome for most superintendents, especially during warmer seasons when creeping bentgrass is prone to heat and drought stress.

Because of its widespread distribution and host range, dollar spot is the most economically significant disease of turfgrass worldwide (Couch, 1995; Hu et al., 2019; Miller et al., 2002). More money is spent in controlling dollar spot than in any other turfgrass disease (Steketee, 2014; Vargas Jr., 2005; Walsh et al., 1999), and it is the most significant disease of home lawns and golf courses (Goodman and Burpee, 1991). In fact, it has been estimated that over 70% of fungicide applications on golf courses go towards controlling three major diseases, one of which is dollar spot (anthracnose and brown patch are the others) (Bonos, 2006). Along these lines, golf course superintendents can sometimes make up to ten fungicide applications in a single year to achieve adequate dollar spot control, which can result in annual costs exceeding $25,000 (Bekken et al., 2022; Hammerschmidt, 2018).

**Dollar spot symptomology and diagnosis**

The initial symptom of dollar spot on individual leaves is the development of yellow-green, chlorotic blotches that have a water-soaked appearance (Couch, 1995). As infection progresses, lesions become white to tan in color and a dark brown or reddish border develops around them. This dark border is common in most turfgrass species but is usually absent in annual bluegrass (Vargas Jr., 2005). Lesions typically extend across the width of the leaf blade as the disease progresses instead of up or down the length of the blade. This causes girdling of the leaf, preventing water and nutrient transport through vascular tissues. This eventually leads to leaf tip dieback or entire leaf blighting and necrosis (Walsh et al., 1999). Lesions themselves often take on the shape of an hourglass, and individual leaves can have one or multiple lesions (Couch, 1995).

Dollar spot symptoms in turfgrass stands vary depending on turfgrass species and management practices. In closely mowed grasses (<2.540 centimeters) of golf courses and athletic fields, the disease is first observed as very small spots of blighted turf. These spots eventually develop into 5.080 to 7.620 centimeter circular, sunken patches of straw-colored, blighted turf (Vargas Jr., 2005). These small, localized patches are often referred to as infection centers. If left untreated, infection centers can coalesce to form irregularly shaped areas of blighted turf (Couch, 1995; Smith, 1955). In higher-mowed grasses of home lawns and other landscapes, dollar spot patches are larger and more irregularly shaped. They can range in size from 15.240 to 30.480 centimeters and can also coalesce to form larger patches if left untreated (Couch, 1995; Smith, 1955).

Fluffy, grayish-white mycelia may be observed in dollar spot-infected turfgrasses during early morning hours when dew is still present on leaves. Extended periods of high humidity and

the presence of dew allows mycelia to grow freely from leaf to leaf (Smiley et al., 1992). However, aerial mycelia of *Clarireedia* spp. may be confused with mycelia of *Pythium* spp. or *R. solani*, so other factors of disease development such as climate, site history, turfgrass species, and symptomology must be considered during disease diagnosis (Walsh et al., 1999). Under a microscope, hyphal morphology can also help distinguish between *Clarireedia*, *R. solani*, and *Pythium* pathogens. *R. solani* hyphae display prominent right-angle branching and constriction at branching origins, while *Clarireedia* hyphae do not. *R. solani* hyphae are often smaller in diameter than *Clarireedia* hyphae as well. *Pythium* hyphae lack septations, which is the easiest diagnostic feature to distinguish it from *Clarireedia*. Additionally, *Pythium* spp. often produce spores, whereas *Clarireedia* spp. do not (Allen et al., 2012).

In culture, *Clarireedia* pathogens produce white, fluffy mycelia and dark brown or black substratal stroma, and they do not produce fruiting structures or spores. There are no reliable morphological features that distinguish one *Clarireedia* spp. from another in culture. Therefore, DNA sequencing is required for species identification. Amplification of *CaM*, *Mcm7*, or *EF-1α* genes, as well as the ITS region, via PCR allows for the identification of species-specific single nucleotide polymorphisms (SNPs) unique to different *Clarireedia* species, as described by Salgado-Salazar et al. (2018), Hu et al. (2019), and Zhang et al. (2022). This process of pathogen isolation, DNA extraction, PCR amplification, and Sanger sequencing for species identification can be time-consuming and expensive. Other useful molecular techniques for *Clarireedia* identification have been developed to help reduce the time and costs associated with these procedures, but each of them still have a few drawbacks of their own. For example, co-dominant cleaved amplified polymorphic sequence (CAPS) and probe-based loop-mediated amplification (LAMP) assays have been developed to rapidly distinguish between *C. jacksonii* and *C.*

*monteithiana* pathogens, but they do not differentiate among the other four *Clarireedia* species (Stackhouse et al., 2024; Stackhouse et al., 2021). Similarly, Groben et al. (2020) developed a quantitative real-time PCR (qPCR) assay to detect and quantify *Clarireedia* in field settings in as little as three hours, but it can only detect pathogen presence and does not distinguish between any *Clarireedia* spp. (Groben et al., 2020).

**Dollar spot epidemiology**

*Clarireedia* overwinters as darkly pigmented stromata on margins of lesions from previous dollar spot outbreaks, or as dormant mycelia in infected grass tissues (Couch, 1995; Fenstermacher, 1970; Walsh et al., 1999). The pathogen can resume growth after overwintering when temperatures reach 16˚C (60˚F). At this temperature, mycelia within infected tissues are able to colonize new foliar tissues (Smiley et al., 1992). During colonization, the pathogen infects turfgrass tissues through stomata or cut leaf tips, and direct penetration into leaves via appressoria occurs as well (Endo, 1966; Monteith and Dahl, 1932). Optimal conditions for dollar spot outbreaks occur at 21 to 27˚C (70 to 80˚F) and when nighttime relative humidity is 85% or higher (Couch, 1995). These conditions correspond to typical spring and fall climates in most regions of the United States, which is why patterns of dollar spot epidemics are usually bimodal, occurring in spring and fall. However, pathogen growth and infection can occur outside of optimal conditions at temperatures ranging from 16 to 30˚C (60 to 86˚F), so epidemics still transpire in warmer summer months if humidity is high enough (Tani and Beard, 1997). Pathogen growth slows considerably at temperatures below 10˚C (50˚F) and above 34˚C (93˚F) (Aynardi et al., 2019; Bennett, 1937). Dollar spot pathogen dissemination over long distances primarily occurs through transportation of infected leaves or debris by people, animals,

equipment, water, and wind (Smiley et al., 1992). *Clarireedia* has also been recovered from commercial seed lots (Rioux et al., 2014), indicating seeds are a possible vessel for pathogen dissemination, especially in cool-season turfgrasses.

Furthermore, certain cultural conditions in turfgrass systems can promote dollar spot outbreaks. Localized dissemination of dollar spot pathogens in a turfgrass stand is favored by prolonged humidity in the turfgrass canopy, as aerial mycelia can utilize the excess moisture on leaf surfaces to progress from plant to plant (Smiley et al., 1992). This is why heavy dew formation in turfgrass stands often intensifies dollar spot severity (Williams, 1996a). Additionally, drought stress favors disease development, as well as excessive thatch buildup (Couch, 1995; Couch and Bloom, 1960). These two conditions are often related, as excessive thatch can prevent water infiltration into the soil, leading to droughty soil conditions. Moreover, nitrogen deficient turfgrasses are more vulnerable to dollar spot outbreaks than those that are adequately fertilized (Burpee and Goulty, 1988; Markland et al., 1969; Williams et al., 1996b). Lastly, closely mowed grasses are generally more susceptible to dollar spot than higher-cut grasses, as close mowing creates denser leaf canopies that facilitate mycelial proliferation (Tani and Beard, 1997).

**Genetic diversity and reproductive strategies of *Clarireedia***

In North America, *Clarireedia* pathogens have no known sexual or diploid stages, and no asexual (conidia) or sexual (ascospores) spore production has been observed. Attempts to produce sporulating structures in laboratory settings have only yielded sterile apothecia (Carbone and Kohn, 1993; Fenstermacher, 1970; Orshinsky and Boland, 2010). Due to the absence of fertile sporulating structures and spores in North American dollar spot strains, it is believed that

mycelia are the only structures produced by these pathogens throughout their life cycle. Therefore, dissemination of mycelium is likely the most important mode of propagation for *Clarireedia* pathogens, meaning local populations are often composed of clones of founding individuals (founder effects) (Hsiang and Mahuku, 1999). Collectively, these characteristics of *Clarireedia* fungi often foster minimal genetic diversity (DeVries et al., 2008).

Much of the work pertaining to dollar spot pathogen diversity has been conducted using vegetative compatibility group (VCG) assays. Vegetatively compatible individuals can fuse their hyphae to form stable heterokaryons, and individuals belonging to the same VCG are more similar to each other than to individuals belonging to different VCGs (Powell and Vargas Jr., 2001). VCGs can represent genetically distinct populations or subdivide populations into groups that can share genetic information via parasexual processes (Carvalho and Mendes-Costa, 2011; Viji et al., 2004). The largest VCG assay conducted for dollar spot pathogens was carried out by Powell and Vargas Jr. (2001). They assessed compatibility among >1300 isolates collected from eight sites in Wisconsin, Michigan, and Illinois and reported six distinct VCGs in total. The vast majority of their isolates fell into three VCGs, indicating little genetic variation within their collection (Powell and Vargas Jr., 2001). Similarly, Deng et al. (2002) reported only four VCGs among 116 isolates collected from southern Ontario and Nova Scotia. Only one of these four VCGs was novel relative to those reported by Powell and Vargas Jr. (2001). Additionally, over half of their isolates belonged to one VCG, once again indicating little diversity within their collection (Deng et al., 2002).

Dollar spot isolates used in the two aforementioned studies were collected from similar locales, which could explain the limited number of VCGs reported. Several studies have shown that isolates collected from different geographic regions are less compatible than isolates

collected from similar regions. For example, in assessing vegetative compatibility among 67 isolates collected from nine U.S. states and Ontario, Viji et al. (2004) reported eleven different VCGs, five of which were new relative to the VCGs reported by Powell and Vargas Jr. (2001). Similarly, Taylor (2010) chronicled nine previously unidentified VCGs among 109 isolates collected in six different countries. Furthermore, Mitkowski and Colucci (2006) evaluated a collection of only 25 isolates from six U.S. states and the United Kingdom and reported eight different VCGs. However, the novelty of these VCGs were unknown because they were not tested against previously identified VCGs (Mitkowski and Colucci, 2006). In contrast to findings from these studies, Sonoda (1989) identified 54 VCGs among 119 dollar spot isolates collected in a single state (Florida). However, all of these isolates came from *Paspalum notatum* (bahiagrass), a $C_4$ grass, whereas most isolates used in the abovementioned studies came from $C_3$ hosts. Reduced vegetative compatibility among $C_4$ dollar spot isolates compared to $C_3$ isolates suggests that host specialization may contribute to genetic diversity in *Clarireedia* (Sonoda, 1989).

Other molecular techniques have also been implemented in a few studies to investigate genetic diversity of *Clarireedia*, often in tandem with VCG assays. Viji et al. (2004) developed amplified fragment length polymorphism (AFLP) markers to assess diversity among a subset (n = 38) of their dollar spot isolates and found that they clustered into two distinct groups, a major group and a minor group. The minor group contained only two isolates collected from bermudagrass in Florida, whereas the major group consisted of 36 isolates collected from various $C_3$ hosts in eight northern U.S. states and Ontario. The authors suggested that this segregation could have been due to host specialization and geographic separation. Within the major group, high genetic similarity was observed among all isolates, as indicated by a similarity coefficient

of .80 (max of 1). Moreover, they also observed correlations between AFLP typing and VCGs, as most of their isolates that had similar AFLP fingerprints belonged to the same VCG. Lastly, their dollar spot collection included several archival isolates gathered from Pennsylvania in the 1970s, as well as contemporary isolates gathered in the 1990s and 2000s from the same region. In assessing the relatedness between these two sets of isolates, the authors discovered that their AFLP fingerprints were quite similar, indicating that the population was probably clonal (Viji et al., 2004). Furthermore, a study conducted by DeVries et al. (2008) using 60 *Clarireedia* isolates collected from Tennessee and Northern Mississippi corroborated many of the findings reported by Viji et al. (2004). Their AFLP fragment analysis revealed high genetic similarity (86 to 100%) among all isolates, and they found that isolates with similar AFLP fingerprints frequently grouped within the same VCG. Additionally, they often found that isolates from the same geographic locations were compatible in VCG assays and clustered together in AFLP analysis. The authors also sequenced conserved genomic regions (carbomoylphospate synthase, translation elongation factor 1-α, β-tubulin, ITS) and reported 100% similarity among all isolates for all regions. Overall, these two studies presented evidence of founder effects and clonality (lack of sexual reproduction) within *Clarireedia* populations (DeVries et al., 2008; Viji et al., 2004).

Furthermore, Raina et al. (1997), used random amplified polymorphic DNA (RAPD) markers to assess genetic variation of *Clarireedia* isolates collected from six U.S. states (n = 25) and Belize (n = 1). Based on RAPD profiles, they found that U.S. isolates clustered into three major groups according to geographical proximity. They also found that isolates collected from the United States showed closer genetic relatedness (>90% similarity) relative to the Belize isolate that was genetically distinct. These results once again convey the impact of spatial

distribution on *Clarireedia* diversity and reinforce the influence of founder effects and clonality in population establishment (Raina et al., 1997). Similarly, Hsiang and Mahuku (1999) also used RADP markers as well as IGS (intergenic spacer region of ribosomal DNA) markers to asses genetic variability among *Clarireedia* isolates gathered from Ontario and Japan. The collection of Ontario isolates (n = 181) came from ten different locations in the southern region of the province, each considered a separate population, while Japanese isolates (n = 10) were collected from various sites all across the country. Japanese isolates were genetically distinct from Ontario isolates, and genetic similarity was higher among Ontario isolates (0.86) compared to Japanese isolates (0.66). Due to the high overall genetic similarity among southern Ontario isolates, the authors suggested a founding population(s) existed in the region. Moreover, most Ontario isolates clustered according to their population of origin, but interestingly, geographically closer populations did not always exhibit greater genetic similarity compared to distant populations. For example, the two populations that were most alike were separated by hundreds of kilometers, while some populations occupying the same county did not cluster together. The authors suggested that this could have been due to migration events between populations, likely facilitated by human activity (i.e. transportation of contaminated equipment or infected sod between locations) (Hsiang and Mahuku, 1999).

Although sex seems to be an insignificant mode of reproduction in *Clarireedia* populations, there have been a few studies that suggest it may be possible for these pathogens. A focal point of one of these studies, performed by Putman et al. (2015), was mating type. In ascomycetous fungi, sexual reproduction is controlled by mating-type genes found at *MAT* loci (Ni et al., 2011). Heterothallic ascomycetes contain a single mating-type locus called *MAT1*, and idiomorphs, which are typically called *MAT1-1* and *MAT1-2*, are alternative sequences at this

locus (Brewer et al., 2011). To be sexually compatible, two individuals must have compatible *MAT1* mating specificities (*MAT1-1* or *MAT1-2*). Moreover, the presence of a 1:1 ratio of *MAT1-1* and *MAT1-2* idiomorphs in a population is indicative of sexual reproductive activity (Amorim et al., 2017). Through examining the distribution of mating types among >1000 *Clarireedia* isolates collected from various locations and hosts around the world, Putman et al. (2015) observed few deviations from this 1:1 idiomorph ratio, suggesting that sex may be possible. Another study performed by Hsiang and Mahuku (1999), some results of which were previously described, also gave some insight into sexual reproduction potential in *Clarireedia*. Upon analyzing molecular profiles generated with RAPD and IGS markers for dollar spot pathogen populations in southern Ontario, they observed low levels of linkage disequilibrium in a few populations. Linkage disequilibrium (LD) is a measure of nonrandom association of alleles at different loci. It is influenced by many different factors including recombination (via sexual reproduction), which tends to breakdown LD (Li and Stephens, 2003). Therefore, the low levels of LD observed in certain pathogen populations led the authors to hypothesize that a combination of sexual reproduction and clonal propagation may contribute to the population structure in this region of Ontario (Hsiang and Mahuku, 1999). Despite the findings from these two studies, morphological data does not yet support the prospect of sexual reproduction in *Clarireedia*, as no sexual (or asexual) spores or spore-producing structures have been observed in North America.

While sexual reproduction may be rare or lacking in *Clarireedia*, heterokaryosis and subsequent parasexual reproduction may serve as mechanisms for these pathogens to generate genetic diversity. Heterokaryosis is defined as the condition in which cells contain more than one genetically distinct nucleus in a common cytoplasm (Caten and Jinks, 1966). It occurs through hyphal anastomosis (hyphal fusion) between two vegetatively compatible individuals and is

integral to VCG assays. The presence of more than one genetically distinct nucleus in a hyphal cell can impart phenotypic plasticity to many asexually reproducing fungi (Kessler et al., 2018; McGuire et al., 2005). The ability of a fungus to exhibit a range of phenotypes (i.e. phenotypic plasticity) can enhance its adaptability to changing environments (James et al., 2008; Sanderson and Srb, 1965; West-Eberhard, 2008). For instance, in their study assessing fungicide adaptability in *Clarireedia*, Kessler et al. (2018) found that the pathogens developed resistance to multiple fungicides through the formation of heterokaryons. Furthermore, heterokaryosis is a prerequisite to parasexual exchange of genetic information, which occurs from the fusion of nuclei (karyogamy) within heterokaryons. Karyogamy does not always occur after hyphal fusion, but if it does, recombinants can arise via mitotic crossing-over or nondisjunction (Käfer, 1961). This recombination can increase genetic diversity. In fact, Zeigler et al. (1997) showed that high levels of fungal diversity could manifest from parasexual reproduction in predominantly asexual populations. Whether parasexual recombination occurs in *Clarireedia* is not known, but it is suspected to occur (Hulvey et al., 2012; Jo et al., 2008b). For example, Liberti et al. (2012) found that 18 of their 47 *Clarireedia* isolates carried both *MAT1-1* and *MAT1-2* nuclei, and after discovering these heterokaryons, they claimed, "These data identify the potential for parasexuality within isolates as a mechanism for generating genetic diversity…".

## Dollar spot management

### *Host Resistance*

As with any plant disease, resistant plant cultivars should be utilized wherever possible in order to prevent or limit infection, especially if there is a history of disease in the area. Most turfgrass managers and researchers rely on variety trial data from the National Turfgrass

Evaluation Program (https://ntep.org) to stay updated on dollar spot resistant turfgrasses. There are no cultivars of any turfgrass species that are completely immune to dollar spot, but a few cultivars exhibit partial resistance (Walsh et al., 1999). The scarcity of highly resistant germplasm for most turfgrass species makes breeding for dollar spot resistance inherently difficult. The fact that many important turfgrasses affected by dollar spot are outcrossers with complex polyploid genomes also makes genetically characterizing host resistance challenging (Stier et al., 2020). Additionally, reliable phenotyping methods outside of subjective visual scoring or digital imaging analysis are lacking, which can further convolute the process of investigating dollar spot host resistance (Sapkota et al., 2022).

Most of the breeding efforts for host resistance to dollar spot are in creeping bentgrass. Creeping bentgrass is the most-widely used species on golf courses in the northern U.S. and Canada, and dollar spot is the most common and persistent disease of this species (Bonos et al., 2006). Past studies have shown wide variation in cultivar susceptibility to dollar spot (Abernathy et al., 2001; Brede, 2007; Golembiewski and Danneberger, 1998; Koch and Kerns, 2012; Settle et al., 2001; Vincelli et al., 1997), and this variation seems to be heavily influenced by environmental conditions (Bonos et al., 2003). Mechanisms of dollar spot resistance in creeping bentgrass are not fully understood, but the inheritance of resistance appears to be quantitative (Bonos et al., 2003; Bonos and Meyer, 2003). A few researchers have conducted mapping studies in creeping bentgrass that have identified quantitative trait loci (QTL) and chromosomal regions that may be of interest for developing resistant cultivars in the future (Chakraborty et al., 2006; Honig et al., 2014). Additionally, a few researchers have employed transgenic breeding approaches in creeping bentgrass, finding that overexpression of a rice thaumatin-like protein

(*TLPD34*) and an *Arabidopsis thaliana* pathogenesis-related protein (*PR5K*) enhanced dollar spot resistance (Fu et al., 2005; Guo et al., 2003).

Research efforts to characterize dollar spot resistance in $C_4$ grasses pale in comparison to creeping bentgrass. The majority of dollar spot resistance work done in $C_4$ grasses has been conducted in seashore paspalum. Seashore paspalum is not as popular as its warm-season bermudagrass and zoysiagrass counterparts, but it is important in areas where a salt-tolerant turfgrass is needed. Steketee et al. (2017) performed dollar spot resistance screening in the field for 90 different seashore paspalum accessions and found that disease severity varied widely among them, with no discrete resistance classes exhibited. In light of this, the authors suggested that dollar spot resistance in seashore paspalum is likely inherited quantitatively, similar to creeping bentgrass (Steketee et al., 2017). Another study conducted by Steketee et al. (2016) aimed to develop an improved screening protocol for dollar spot resistance in seashore paspalum. Across two growing seasons, the authors tested five different *Clarireedia* isolates against five different seashore paspalum genotypes and found no significant interaction between plant genotype and pathogen isolate. The group concluded that seashore paspalum resistance genes are likely not isolate specific and using just one highly virulent dollar spot isolate may be sufficient to screen for host resistance in seashore paspalum (Steketee et al., 2016). In another study assessing host resistance in a different *Paspalum* species, Williams (2005) found that two tetraploid bahiagrass cultivars, ‘Argentine’ and ‘Tifton 7’, were significantly less susceptible to dollar spot compared to several other cultivars tested (Williams, 2005).

While utilizing genetic resistance is an important dollar spot control strategy, it is worth noting that turfgrass managers often do not have the luxury of readily switching to new resistant cultivars. Switching to a new cultivar usually requires full renovation of a turfgrass stand, which

is a time consuming and costly endeavor, especially on larger sites such as golf courses and athletic fields (Esponda, 2019). In the short-term, spending money on equipment, products, and labor to treat disease is usually more cost effective than renovation. However, over time, transitioning to resistant cultivars may be more cost effective and environmentally sustainable, so long as resistance is durable. The durability of dollar spot resistance is not yet known for any turfgrass species or cultivar but remains a focal point of many turfgrass breeding programs (Sapkota et al., 2022). Despite the current challenges associated with developing or adopting resistant cultivars, ongoing research to better understand the mechanisms of dollar spot host resistance will provide turfgrass managers and researchers with better guidance in utilizing genetic disease control strategies.

***Chemical management***

Chemical management remains the most important aspect of dollar spot control. Nonchemical options rarely provide complete control of the disease, but they do reduce disease pressure, thereby indirectly improving fungicide performance. Currently, there are over 150 fungicides registered for dollar spot and over 40 active ingredients or active ingredient combinations (Martinez-Espinoza, 2021). According to the Fungicide Resistance Action Committee, the four main fungicide classes used to control dollar spot include benzimidazoles, demethylation inhibitors (DMIs), dicarboximides, and succinate dehydrogenase inhibitors (SDHIs) (FRAC, https://www.frac.info/home). Fungicides within these classes are systemic (single-site) and can have preventative or curative action. Chlorothalonil, a benzonitrile that is not part of these fungicide groups, is the most important multi-site fungicide (contact, preventative) used for dollar spot control. It is often tank-mixed with systemic fungicides in order to delay, avoid, or manage fungicide resistance (Latin, 2011). Furthermore, because dollar

spot infection can occur over an entire growing season, it frequently coincides with other turfgrass disease outbreaks. Therefore, pesticide applicators often tank-mix and apply multiple fungicides with different modes of action to provide joint control of both dollar spot and other diseases (Latin, 2011).

Application scheduling is arguably the most important facet of controlling dollar spot with fungicides. The best approach for timing fungicide applications is to anticipate outbreaks and apply fungicides before symptoms occur. In other words, early or preventive fungicide applications work best for dollar spot control, so constant monitoring of turfgrass stands for indications of disease is of utmost importance. Because dollar spot epidemics are more likely to occur in mild climatic conditions, the preventative window for fungicide application typically coincides with daytime air temperatures steadying at 21˚C (70˚F) (Couch, 2000). During this time, depending on the fungicide(s) used, applications are usually made at low label rates over 7 to 10 day or 14 to 21 day intervals. If applications are made after symptoms are observed, high label rates may be used at 5 to 7 day intervals (Couch, 2000). After initial dollar spot outbreaks, fungicide applications are typically made on a biweekly basis while conditions remain favorable for the disease.

A few weather-based models that attempt to predict the occurrence of dollar spot epidemics have been developed to help reduce fungicide application frequencies (Hall, 1984; Ryan et al., 2012; Smith, 2013; Smith et al., 2018). These models assist turfgrass managers in eliminating wasteful fungicide applications by providing them with precise treatment time frames. The most recent model developed by Smith et al. (2018) uses five-day moving averages of daily relative humidity and daily average air temperature to generate a probability of dollar spot outbreak occurring on any given day. The model has been validated through years of field

research in Wisconsin, and could potentially reduce fungicide use by up to 30% (Smith et al., 2018). Further validation of this model and others like it in different regions of the country may help reduce fungicide resistance and reliance (Smith et al., 2018).

***Fungicide resistance***

The first report of fungicide resistance for any turfgrass disease occurred in 1966, when Jackson (1966) found evidence that dollar spot pathogens were resistant to cadmium fungicides. These fungicides had been used for disease control in turfgrass systems since the 1940s but were banned in the U.S. in the late 1980s (National Toxicology Program, 2021). The first account of dollar spot resistance to a modern-day fungicide class occurred in 1973. Through *in vitro* sensitivity assays involving benzimidazole fungicides, Goldenberg and Cole (1973) found that several of their isolates collected from golf courses in New Jersey, Illinois, Ohio, and Pennsylvania were up to 100 times more tolerant to benomyl than sensitive isolates. Since then, accounts of dollar spot resistance or insensitivity to the benzimidazoles have been numerous (Burpee, 1997; Ghimire et al., 2023; Koch et al., 2009; Putman et al., 2010; Warren et al., 1974). Likewise, widespread resistance to the dicarboximides (Bishop et al., 2008; Detweiler et al., 1983; Kim et al., 2010; Mocioni et al., 2011) and the DMIs (Detweiler et al., 1983; Ghimire et al., 2023; Golembiewski et al., 1995; Hsiang et al., 2007; Miller et al., 2002) has been reported since the 1980s. As a newer class of fungicides released for dollar spot control in the 2000s, SDHI resistance is not as widespread relative to these other classes. However, isolated cases of SDHI resistance or insensitivity have been documented (Anthony and Kerns, 2017; Popko et al., 2018; Sang et al., 2015; Zhang et al., 2021).

Complicating matters, dollar spot pathogens often exhibit cross resistance (i.e. resistance to more than one fungicide within the same chemical class) or multiple resistance (i.e. resistance

to different fungicide classes) (Jo et al., 2008a). The first account of dollar spot cross resistance to modern-day fungicides occurred in 1974, when Warren et al. (1974) reported that several isolates in their collection exhibited normal growth on PDA media amended with benomyl and other benzimidazoles. Cross resistance has frequently been reported in *Clarireedia* populations since then (Doney Jr. and Vincelli, 1993; Golembiewski et al., 1995; Hsiang et al., 1997; Ok et al., 2011; Zhang et al., 2021). While cross resistance remains a significant concern for many turfgrass managers, the existence of multiple resistance is generally more threatening in terms of chemical management due to the failure of multiple modes of action. Detweiler et al. (1983) were the first to report dollar spot multiple resistance to modern fungicides after detecting resistance to both iprodione (dicarboximide) and benomyl (benzimidazole) in *in vitro* and greenhouse assays. More recently, Stephens and Kaminski (2019) found that 585 (86%) of their 681 isolates collected from Pennsylvania golf courses exhibited a profile in which they were resistant or insensitive to at least two different fungicide classes included in their *in vitro* sensitivity assays. Several other studies like these have also reported the presence of multiple resistance in *Clarireedia* populations (Bishop et al., 2008; Jo et al., 2006; Koch et al., 2009; Ok et al., 2011; Putman et al., 2010; Sang et al., 2019).

***Cultural management***

Fertilization is one of the most important cultural practices of turfgrass management. Turfgrasses require nitrogen in larger amounts than any other mineral element, and increasing rates of N typically decrease dollar spot severity (Townsend et al., 2021). The mechanisms of dollar spot suppression through N fertilization are unknown, but three theories have been proposed: 1) increased plant growth resulting from N fertilization strengthens the plant to ward off disease or escape the pathogen, 2) the buildup of microbial populations in the soil or

phylloplane as a result of N fertilization leads to disease suppression, 3) changes in pH caused by N fertilization changes pathogen virulence or plant resistance (Townsend et al., 2021). Additionally, increased turfgrass growth resulting from N fertilization leads to more mowing events that can help remove necrotic tissues that serve as *Clarireedia* nutrient or inoculum sources (Couch, 1995; Walsh et al., 1999).

Several studies have shown that N applications can directly suppress dollar spot (Cook et al., 1964; Golembiewski and Danneberger, 1998; Landschoot and McNitt, 1997; Markland et al., 1969; Sartain and Dudeck, 1980; Williams et al., 1996b). Markland et al. (1969) proposed that disease suppression through N fertilization was due to the increased utilization of carbohydrate reserves in plant tissues, which allows plants to essentially outgrow pathogens (Markland et al., 1969). Moreover, Liu et al. (1995) applied different amendments, including several different N fertilizers, to creeping bentgrass greens over a three year span. They observed a significant buildup of soil microbes in areas fertilized with N and noticed decreased dollar spot severity in these areas. They suggested that antagonistic effects on pathogens from microbial organisms could have been responsible for reduced dollar spot (Liu et al., 1995). Ryan et al. (2011) found that decreased pH caused by ammonium sulfate applications slightly decreased dollar spot severity in creeping bentgrass, but these results contradict with Markland et al. (1969), who observed no significant effects in disease development at different soil pH levels. While N fertilization typically decreases dollar spot severity, excess N can promote diseases such as brown patch, *Bipolaris* leaf spot, *Pythium* blight, take-all patch, *Microdochium* patch, and gray leaf spot (Turner and Hummel Jr., 1992). Therefore, in order to maintain appropriate levels of N, fertilization is best tailored according to species of turfgrass being grown, growth stage, weather patterns, and soil type and condition.

In comparison to N fertilization assessments, far fewer studies have been performed in evaluating the impacts of phosphorus and potassium fertility on dollar spot development. Juska and Murray (1974) reported that dollar spot damage was less severe on bermudagrass plots treated with K when compared to non-treated controls. Pritchett and Horn (1966) also found that K fertilization decreased dollar spot severity in bermudagrass. However, Bier et al. (2018) and Woods et al. (2006) reported that dollar spot was not affected by different K fertilizer treatments on creeping bentgrass greens. Waddington et al. (1978) also found that K fertilization had no effect on dollar spot severity in creeping bentgrass. Little is known about the effects of phosphorus alone on dollar spot development, but phosphorus deficiencies could lead to reduced vigor that escalates disease issues (Christians et al., 1979; Johnson et al., 2003). Maintaining phosphorus fertility levels that are properly balanced with N and K levels can stimulate growth and recovery that curb disease pressure (Couch, 2000; McDonald et al., 2019).

Several other cultural practices can be effective in mitigating dollar spot incidence and/or severity. An important practice often implemented on golf courses is the removal of dew from grass in the early morning hours (Williams, 1993). Dew primarily consists of guttation exuded from leaf hydathodes, and this guttation is rich in carbohydrates and amino acids that serve as nutrients for *Clarireedia* fungi. The removal of dew is usually done by mowing or dragging poles or hoses across the grass surface. Turfgrass managers also spray water on dew-covered grass to remove it. This practice may seem counterintuitive, but the force of spraying water physically displaces guttation from leaf surfaces (Vargas Jr., 2005). Moreover, drought stress can increase dollar spot severity, so regimented irrigation to keep soil moisture levels at field capacity is recommended. Irrigation in the early morning allows plants to dry out during the day and promotes better water usage and absorption, all of which reduce disease pressure. Late

evening irrigation increases drying times and periods of leaf wetness, which makes turfgrass stands more susceptible to dollar spot infection (Allen et al., 2012). Other practices, such as pruning or removing adjacent trees and shrubs, also promote more air circulation to decrease drying times in turfgrass stands (Vargas Jr., 2005). Additionally, many golf course superintendents install greenside fans to increase air circulation and reduce drying times.

Mowing turfgrasses at improper heights facilitates stresses that could incite dollar spot. Proper mowing height depends on the species and cultivar of turfgrass used. The collection and disposal of grass clippings after mowing removes secondary *Clarireedia* inoculum, which could lead to reduced dollar spot incidence (Walsh et al., 1999). Sharp mower blades promote cutting, rather than tearing, of leaf blades during mowing events. Tearing of the leaf blade can create more surface area for *Clarireedia* pathogens to infiltrate (Emmons and Rossi, 2015). However, Ellram et al. (2007) found no significant differences in dollar spot incidence and severity from using sharp or dull mower blades in creeping bentgrass. A practice that is commonly performed in tandem with mowing, at least on golf courses and athletic fields, is rolling. Rolling is used to firm and smooth turfgrass surfaces, and some studies have demonstrated that this practice alone can reduce dollar spot severity (Espevig et al., 2020; Genova et al., 2016; Giordano et al., 2012a; Giordano et al., 2012b).

Furthermore, excess thatch removal is also an important cultural management practice to promote water infiltration and prevent droughty soil conditions, thereby alleviating dollar spot disease pressure (Wagner and Halisky, 1981). Decreasing or removing thatch is often done by coring, vertical mowing, or topdressing. Apart from decreasing thatch, sand topdressing alone has been shown to significantly reduce dollar spot infection, sometimes by up to 50% (Green et al., 2019; Skorulski et al., 2010). Green et al. (2019) suggested that sand topdressing may dilute

dollar spot stroma in the soil, reducing inoculum loads. Finally, compacted soils lead to plant stress that may increase susceptibility to dollar spot. Therefore, routine cultivation and aeration is recommended to alleviate compaction (Allen et al., 2012).

***Biological management***

In the context of plant pathology, Pal and Gardener (2006) define biological control as "the use of microbial antagonists to suppress diseases." Extensive research has been conducted in assessing the efficacy of biological products or agents in controlling dollar spot. No standalone biological product completely eradicates dollar spot, but some may alleviate disease pressure. Goodman and Burpee (1991) found that applications of *Fusarium heterosporum*-infested sand-cornmeal topdressing on creeping bentgrass greens suppressed dollar spot by up to 93% compared to untreated control plots. Similarly, applications of pelletized *Gliocladium virens* resulted in a 50% decrease in dollar spot severity in bermudagrass field trials (Haygood and Mazur, 1990). Nelson and Craft (1991) found that topdressings infested with *Enterbacter cloacae* bacteria suppressed dollar spot by up to 63% in creeping bentgrass and annual bluegrass greens. Lo et al. (1997) reported up to 71% control of dollar spot from *Trichoderma harzianum* (strain 1295-22) applications in creeping bentgrass field trials conducted over four years. Another *Trichoderma* species, *Trichoderma atroviride*, has also shown promise in suppressing dollar spot (Coelho et al., 2021). Moreover, Kabbage et al. (2020) found that poacic acid, a secondary metabolite produced by several grass species, reduced dollar spot in the field by up to 67% and inhibited *C. jacksonii* growth *in vitro* of by up to 93%. Furthermore, hypovirulent strains, which are strains with reduced ability to infect susceptible hosts, of *Clarireedia* have also shown potential in effectively suppressing disease. Zhou and Boland (1998) applied hypovirulent *Clarireedia* strains to creeping bentgrass swards that were artificially inoculated with virulent

strains and found that one hypovirulent isolate, Sh12B, suppressed disease by up to 80% for an entire year. They also found that the same isolate suppressed dollar spot by up to 58% in naturally infected creeping bentgrass plots (Zhou and Boland, 1998).

Other studies have demonstrated the potential of endophytes in suppressing dollar spot, primarily in $C_3$ turfgrasses. Endophytes are microorganisms that reside in plant tissues for at least part of their life cycle without causing disease to their host (Hyde et al., 2019). In creeping bentgrass greenhouse trials, Shehata et al. (2016) found that plants treated with several bacterial endophytes (*Burkholderia gladioli* isolates) of ancient and wild *Zea* spp. significantly reduced dollar spot severity compared to untreated controls. Similarly, in field trials, Clarke et al. (2006) found that strong creeping red fescue (SCRF; *Festuca rubra* subsp. *rubra*) harboring the fungal endophyte *Epichloe festucae* exhibited significantly less dollar spot severity compared to endophyte-free SCRF, reporting 61% less disease on average over three years. Tian et al. (2017) later characterized an antifungal protein of *E. festucae* called *Efe*-AfpA and discovered it had *in vitro* inhibitory activity against *Clarireedia*. Given this, the authors suggested that *Efe*-AfpA may contribute to dollar spot resistance in *E. festucae*-infected turfgrasses (Tian et al., 2017).

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# CHAPTER 2

# GENETIC DIVERSITY AND POPULATION STRUCTURE OF *CLARIREEDIA MONTEITHIANA*, CAUSAL AGENT OF DOLLAR SPOT, IN GEORGIA TURFGRASSES

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**Abstract**

Dollar spot is one of the most detrimental ailments of turfgrass worldwide. Despite the global prevalence, economic significance, and prolonged history of the disease, the taxonomic classification of dollar spot pathogens was not resolved until 2018. The placement of dollar spot fungi into the novel *Clarireedia* genus provides a new framework in which to explore pathogen biology and evolution. This study aimed to investigate population structure and genetic diversity of *Clarireedia* in Georgia turfgrasses. An original collection of 210 dollar spot isolates was obtained from eleven turfgrass species and 145 counties across the state from 2019 to 2023. *C. monteithiana* was found to be the predominant species causing the disease, comprising 96% of our isolate collection. Based on single nucleotide polymorphisms derived from a genotyping-by-sequencing approach, population structure analyses for 149 *C. monteithiana* isolates revealed two distinct populations. Although the populations varied in size, they displayed similar genetic diversity. No associations were observed between population structure and year of collection, sampling location, or host species. Moreover, index of association tests for both populations suggested they were clonal. Overall, this study is the first to utilize a next generation sequencing technique for a collection of *C. monteithiana* isolates and revealed population structure and genetic diversity of the dollar spot pathogen in Georgia, despite indications of its clonal reproduction. These findings may offer insight into pathogen biology and adaptation, which could aid turfgrass practitioners in making more informed disease management decisions.

**Keywords**: Dollar spot, *Clarireedia*, Turfgrass, Genetic Diversity, Population Structure

## Introduction

Dollar spot is the most widespread turfgrass disease in North America and can occur on nearly all cultivated cool- and warm-season turfgrass species (Smiley et al., 1992). It reduces turfgrass quality and playability, and each year in the United States, more money is spent on the chemical management of dollar spot than any other turfgrass disease (Steketee et al., 2017; Vargas Jr., 2005). Individual leaves of affected turfgrass plants often develop white to straw-colored lesions bounded by reddish-brown borders (Allen et al., 2012). These lesions usually expand across the width of the leaf blade, leading to girdling, blighting, and dieback (Sapkota et al., 2022; Walsh et al., 1999). As infection progresses throughout a turfgrass stand, disease symptoms typically manifest as small, circular, localized patches of blighted turfgrass (Couch, 1995). These patches are commonly referred to as infection centers, and in closely mowed turfgrass stands, they often appear sunken and can range from two to three inches in diameter (Monteith and Dahl, 1932). In higher-mowed turfgrass stands, dollar spot infection centers are more irregularly shaped and larger, usually ranging from four to twelve inches in diameter (Monteith and Dahl, 1932). If left untreated, infection centers can coalesce to form larger areas of blighted turfgrass (Smith, 1955).

*Sclerotinia homoeocarpa* was formerly described as the causal agent of dollar spot (Bennett, 1937), but a recent multilocus phylogenetic study conducted by Salgado-Salazar et al. (2018) placed dollar spot pathogens into a newly formed genus, *Clarireedia*. The group identified four species within the *Clarireedia* genus including *C. homoeocarpa*, *C. benettii*, *C. jacksonii*, and *C. monteithiana*. Both *C. homoeocarpa* and *C. benettii* infect cool-season turfgrasses and are mostly distributed throughout the United Kingdom. *C. monteithiana* and *C. jacksonii* have a global distribution and are the most prevalent dollar spot pathogens in the

United States. *C. monteithiana* primarily infects warm-season turfgrasses, whereas *C. jacksonii* primarily infects cool-season turfgrasses (Salgado-Salazar et al., 2018). Furthermore, shortly after this taxonomic reclassification, three additional dollar spot pathogen species were identified: *C. paspali*, *C.* aff. *paspali*, and *C. hainanense* (Hu et al., 2019; Zhang et al., 2022). These species were collected from seashore paspalum (*Paspalum vaginatum* Swartz) in China, and *C.* aff. *paspali* has also been recovered from seashore paspalum in Hawaii (Bahri et al., 2023). The seven aforementioned *Clarireedia* species are differentiated by specific sequence variations in the rDNA internal transcribed spacer (ITS) region, as well as in calmodulin (*CaM*), DNA replication licensing factor Mcm7 (*Mcm7*), and translation elongation factor 1-α (*EF-1α*) genes (Hu et al., 2019; Salgado-Salazar et al., 2018; Zhang et al., 2022).

Studies pertaining to the genetic diversity of dollar spot pathogens have mostly involved isolates sampled from cool-season turfgrasses and have primarily been conducted using vegetative compatibility group (VCG) assays (Deng et al., 2002; Mitkowski and Colucci, 2006; Powell and Vargas Jr., 2001; Taylor, 2010; Viji et al., 2004). These VCG studies report relatively low levels of genetic diversity within *Clarireedia* spp. Powell and Vargas Jr. (2001) reported six VCGs among 1,332 isolates collected from Wisconsin, Michigan, and Illinois. Similarly, Deng et al. (2002) reported four VCGs among 116 isolates collected from southern Ontario and Nova Scotia, and only one of the four VCGs was novel relative to the ones established by Powell and Vargas Jr. (2001). Furthermore, others who have utilized molecular markers, such as random amplified polymorphic DNA (RAPD) (Hsiang and Mahuku, 1999; Raina et al., 1997), amplified fragment length polymorphisms (AFLP) (DeVries et al., 2008; Viji et al., 2004), and inter-simple sequence repeats (ISSR) (Jo et al., 2008a), have also reported low levels of genetic variability within *Clarireedia* spp. For example, using AFLP (DeVries et al.,

2008) and RAPD markers (Hsiang and Mahuku, 1999), 86% to 100% similarity was observed among 60 dollar spot isolates collected from ten locations across Tennessee and northern Mississippi, and among 181 isolates acquired from ten locations across southern Ontario. The lack of genetic diversity observed in these studies is likely attributed to the presumed absence of sexual reproduction in dollar spot pathogen populations. In North America, no spore production (asexual or sexual) has been observed for *Clarireedia* fungi (Salgado-Salazar et al., 2018), and attempts to produce sporulating structures in laboratory settings have only yielded sterile apothecia (Carbone and Kohn, 1993; Fenstermacher, 1970; Orshinsky and Boland, 2010). Due to the absence of fertile sporulating structures and spores in North American dollar spot strains, it is believed that hyphae and stromata are the only structures these pathogens produce throughout their life cycle. Therefore, dissemination of mycelium is likely the most important mode of propagation for *Clarireedia* pathogens, meaning local populations are often composed of clones of founding individuals (i.e. founder effects) (Hsiang and Mahuku, 1999).

Despite the lack of morphological data supporting the prospect of sexual reproduction, a few studies have indicated that it still may be possible for these pathogens. By examining the distribution of mating types for dollar spot isolates (n = 1,019) acquired from various cool- and warm-season hosts around the world, Putman et al. (2015) found few departures from a 1:1 ratio of *MAT1-1* and *MAT1*-2 idiomorphs within several *Clarireedia* populations, aligning with what would be expected in sexually reproducing ascomycetous fungi. Similarly, Hsiang and Mahuku (1999) used RAPD marker profiles to assess gametic linkage disequilibrium in eight *Clarireedia* populations located in southern Ontario and found that half of them exhibited low levels of disequilibrium, indicating the potential for random mating. In addition to the possibility of sexual reproduction, heterokaryosis and subsequent parasexual recombination may also serve as

mechanisms for *Clarireedia* pathogens to generate genetic diversity. While heterokaryosis has been observed in *Clarireedia* through numerous VCG studies, Jo et al. (2008b) were the first to utilize nitrate-nonutilizing mutants, which offer more accurate detection of vegetative compatibility, to clearly verify its occurrence. Moreover, although it has not been experimentally confirmed in pathogen populations, several researchers suspect parasexual recombination may occur in *Clarireedia* due to the potential for heterokaryosis and its requisite role in the parasexual cycle (Hulvey et al., 2012; Jo et al., 2008b; Kessler et al., 2018; Liberti et al., 2012).

Utilizing next generation sequencing (NGS) technology could provide a more accurate appraisal of pathogen genetic diversity. Genotyping-by-sequencing (GBS) is a genome-wide, high-throughput, cost-effective NGS technique used for single nucleotide polymorphism (SNP) discovery and genotyping (Elshire et al., 2011). It has been successfully used to assess population structure and genetic variability of several fungal pathogens including *Alternaria* spp. (Adhikari et al., 2019; Adhikari et al., 2024), *Puccinia triticina* (Aoun et al., 2020), and *Fusarium oxysporum* (Halpern et al., 2020). However, to our knowledge, no previous research has implemented GBS or any other NGS techniques to investigate the diversity and structure of dollar spot pathogens, nor have there been any diversity studies conducted since the taxonomic reclassification of these pathogens in 2018 (Salgado-Salazar et al., 2018). Furthermore, while studies assessing the variability of dollar spot isolates from warm-season turfgrasses are far less common than those involving isolates from cool-season turfgrasses, they have revealed higher levels of genetic dissimilarity (Liberti et al., 2012; Sonoda, 1989). Additionally, dollar spot isolates collected from the state of Georgia have never been represented in any prior research relating to pathogen structure and diversity.

The main goal of the current study is to elucidate the population structure and genetic diversity of *Clarireedia* spp. in Georgia. Given the prevalence of warm-season turfgrasses across Georgia landscapes, we hypothesize that *C. monteithiana* may be the most widespread causal agent of dollar spot in the state. The specific objectives of this study were to 1) generate a collection of *Clarireedia* spp. isolates sampled from various locations throughout the state of Georgia and identify the predominant species causing dollar spot on turfgrass; 2) determine the population structure of collected isolates using a GBS approach; 3) characterize the genetic diversity of *Clarireedia* spp. populations; and 4) identify potential signatures of heterokaryosis or recombination.

## Materials and methods

### Dollar spot sample collection and species identification

A total of 210 dollar spot samples were collected from various general landscapes (e.g. parks, schools, businesses, community landscapes, public grounds), residential areas, athletic fields, sod farms, universities, and golf courses across the state of Georgia from 2019 to 2023. Samples were acquired from 145 different Georgia counties and eleven different grass hosts, including eight warm-season hosts—bahiagrass (*Paspalum notatum*), bermudagrass (*Cynodon* spp.), carpetgrass (*Axonopus fissifolius*), centipedegrass (*Eremochloa ophiuroides*), crabgrass (*Digitaria* spp.), seashore paspalum (*Paspalum vaginatum*), St. Augustinegrass (*Stenotaphrum secundatum*), and zoysiagrass (*Zoysia* spp.)—and three cool-season hosts—annual ryegrass (*Lolium multiflorum*), creeping bentgrass (*Agrostis stolonifera*), and tall fescue (*Festuca arundinacea*). *Clarireedia* pathogens were isolated from samples following protocols from Sapkota et al. (2020). Briefly, symptomatic turfgrass tissues were cut into 2-cm$^2$ pieces under

sterile conditions and surface disinfected. Surface disinfection procedures included a 2-minute soak in 0.8% sodium hypochlorite, followed by a 2-minute soak in 80% ethanol, and finally a rinse with sterile water three times. Tissues were then left to dry for five minutes and plated onto potato dextrose agar (PDA) media. Plates were sealed and incubated at room temperature under 12-hour light. Hyphae began to emerge from tissues ~2 to 3 days after plating, and after microscopically confirming the presence *Clarireedia*, hyphal tips were then transferred to fresh PDA plates to attain pure cultures. After obtaining pure cultures, isolates were stored in Microbank vials (Pro-Lab Diagnostics, CA) at -80˚C and in sterile grain mixtures of oat, barley, and wheat at -20˚C.

Species identification of dollar spot isolates was executed by first extracting genomic DNA from seven-day old pure *Clarireedia* cultures using a CTAB method (Doyle and Doyle, 1987). DNA quality and concentration were checked using 1% agarose gel electrophoresis and a NanoDrop spectrophotometer (NP80; Implen, U.S.). After DNA extraction and quality checks, the ITS region of each isolate was amplified via PCR using an ITS4/ITS5 primer set (White et al., 1990). Each PCR reaction contained 0.5 µM of each primer, approximately 30 ng genomic DNA, and 1X GoTaq Green Master Mix (M712B; Promega Corporation, Madison, WI) in a final reaction volume of 20 µl. Moreover, thermal cycler (SimpliAmp Thermal Cycler A24812; Thermo Fisher Scientific Inc., U.S.) conditions for amplification included: initial denaturation at 95˚C for 5 minutes; 30 cycles of denaturation at 95˚C for 35 seconds, annealing at 57˚C for 35 seconds, and extension at 72˚C for 1 minute; and a final extension of 72˚C for 10 minutes. Amplification of the ITS region was confirmed by 2% agarose gel electrophoresis. After amplification, PCR products were sent to Eurofins Genomics (KY, U.S.) for purification and Sanger sequencing, and resulting sequences were then blasted against the GenBank nucleotide

database (NCBI) to affirm genus-level identification of *Clarireedia*. To ascertain species-level identification of *Clarireedia* isolates, our ITS sequences were aligned against the ITS sequences of *C. monteithiana* (GenBank accession 'KF545306'), *C. jacksonii* (GenBank accession 'MF964320'), *C. benetti* (GenBank accession 'KF545316'), *C. homoeocarpa* (GenBank accession 'MF964322'), *C. paspali* (GenBank accession 'MH392074'), and *C.* aff. *paspali* (GenBank accession 'MH392062') references from Salgado-Salazar et al. (2018) and Hu et al. (2019). Alignment was performed in MEGA X software using the ClustalW algorithm (Kumar et al., 2018), and species designation was determined by manually inspecting species-specific SNP combinations that each isolate possessed according to reference sequences (Hu et al., 2019; Salgado-Salazar et al., 2018).

**Genotyping-by-sequencing (GBS), SNP calling, and data filtering procedures**

Due to the low number of *C. jacksonii* isolates identified (n = 8), only *C. monteithiana* isolates (n = 202) were included in the GBS population genetic structure analysis. Using genomic DNA (≥100 ng) of the *C. monteithiana* isolates, GBS libraries were prepared by the University of Wisconsin Biotechnology Center according to protocols from Elshire et al. (2011). Briefly, DNA was digested with *Pst*I and *Msp*I restriction enzymes, barcode adapters were ligated to the ends of restriction fragments, adapter-ligated fragments were pooled and amplified via PCR, and PCR products were purified. Paired-end (2 × 150 bp) library sequencing was then performed using an Illumina NovaSeq6000 sequencing platform (Illumina, San Diego, CA).

Raw GBS sequence data was processed by the University of Wisconsin Bioinformatics Resource Center using the TASSEL-GBS v2 pipeline (Glaubitz et al., 2014). Prior to running the pipeline, quality control measures were implemented by using Skewer software (Jiang et al.,

2014) to trim adapters, primers, and low-quality bases to achieve minimum Phred scores of 20. After quality control steps, the TASSEL GBSSeqToTagDBPlugin (kmerLength = 64, minKmerL = 20, mxKmerNum = 100000000) was used to convert raw sequence data to a unique tag database. Tags were then exported in FASTQ format using the TagExportToFastqPlugin and aligned to an in-house *C. monteithiana* reference genome (isolate 'DS9'collected from bermudagrass in Spalding County, GA, in 2019; PacBio-Illumina sequenced, 46.26 Mb, 106 contigs) using the Burrows-Wheeler alignment method (--very-sensitive) in Bowtie 2 (Langmead and Salzberg, 2012). The resulting sequence alignment map (SAM) file was imported into the GBS database via the SAMToGBSdbPlugin (minMAPQ = 10, aProp = 0, aLen = 20), and SNPs were identified from aligned sequence data using the DiscoverySNPCallerPluginV2 with a minimum minor allele frequency threshold of 0.01 (mnMAF = 0.01). The ProductionSNPCallerPluginV2 (kmerLength = 64) was then used to process and convert aligned sequence data and identified SNPs into Variant Call Format (VCF). Further filtering was conducted to retain only homozygous biallelic sites with a read depth of ≥4. Isolates and markers with more than 20% missing data were also filtered out.

**Population structure and genetic diversity analyses**

The R v4.3.2 package *poppr* (Kamvar et al., 2014) was first utilized to identify the number of unique multi-locus genotypes (MLGs) among *C. monteithiana* isolates, as well as to generate a genotype accumulation curve to determine the minimum number of loci needed to differentiate individuals. Using the identified MLGs, the Bayesian model-based clustering method in STRUCTURE v2.3.4 software was employed to infer population structure (Pritchard et al., 2000). Parameters for population structure inference included K (number of simulated

populations) values ranging from K = 1 to K = 8, with ten independent runs conducted for each K. Additionally, each run was executed with a burn-in period length of 100,000, along with 100,000 Monte Carlo Markov Chain replications after burn-in. After running STRUCTURE, the number of optimal K was determined using StructureSelector (Li and Liu, 2018), and individuals were then grouped by population based on a membership probability threshold of 0.90. To confirm and visualize the population structure of *C. monteithiana* isolates, a phylogenetic analysis was carried out using the weighted neighbor-joining method (Saitou and Nei, 1987) (1000 bootstrap repetitions) in Darwin v6.0.21 (Perrier and Jacquemond-Collet, 2006), as well as a principal coordinate analysis (PCoA) (999 bootstrap repetitions) using GenAlEx v6.51b2 (Peakall and Smouse, 2006). The constructed PCoA plot was also used to evaluate potential associations based on the year and turfgrass host from which isolates were collected.

Several genetic diversity indices were computed by genetic population as identified by STRUCTURE using GenAlEx and the *poppr* package in R, including the number of effective alleles (Ne), number of private alleles (Pa), Shannon's information index (I), unbiased diversity (uh), percentage of polymorphic loci (%P), and expected number of MLGs (based on rarefaction analysis) (eMLG). Mantel tests were also conducted in GenAlEx to determine if there was a correlation between geographic (natural log-transformed) and genetic distances for all *C. monteithiana* isolates, as well as within individual populations. Furthermore, the gene flow (*Nm*) between populations was estimated using GenAlEx, and based on this estimate, the following formula was utilized to calculate the coefficient of genetic differentiation ($G_{ST}$) between populations: $Nm = 0.5(1-G_{ST})/G_{ST}$ (McDermott and McDonald, 1993). To provide insight into the reproductive strategy of *C. monteithiana*, the index of association ($I_A$) and standardized index of association ($r\bar{d}$) were calculated for each population using the *poppr* package. Lastly, a neighbor-

net phylogenetic network (Bryant and Moulton, 2004) was constructed using GBS marker data in SplitsTree v6.3.41 to detect signals of recombination (Huson and Bryant, 2006).

## Results

### *Clarireedia* species identification based on ITS sequenced region

A total of 210 dollar spot isolates collected across the state of Georgia were characterized to the species level through DNA sequencing of the ITS region (Table S2.1). *C. jacksonii* and *C. monteithiana* were the only two species identified among the entire collection of isolates, with *C. monteithiana* (n = 202) comprising the majority (96%) of the collection. *C. jacksonii* was recovered from six different Georgia counties, and *C. monteithiana* was recovered from 140 counties. Of the *C. monteithiana* isolates, all but three were obtained from warm-season hosts. Most *C. monteithiana* isolates were gathered from bermudagrass (n = 133), but other hosts included zoysiagrass (n = 26), bahiagrass (n = 10), centipedegrass (n = 16), seashore paspalum (n = 4), St. Augustinegrass (n = 4), carpetgrass (n = 1), crabgrass (n = 5), creeping bentgrass (n = 2), and tall fescue (n = 1). *C. jacksonii* comprised 4% of the entire isolate collection (n = 8), with four isolates obtained from both cool- and warm-season hosts. Specifically, *C. jacksonii* hosts included creeping bentgrass (n = 1), annual ryegrass (n = 3), bermudagrass (n = 3), and St. Augustinegrass (n = 1). Furthermore, 50 isolates within the collection possessed an intron (420 bp) located at the 3' end of the small subunit (SSU) rDNA region. This intronic region was found exclusively in *C. monteithiana* isolates (Figure S2.1).

**GBS reads and SNP calling**

A total of 202 *C. monteithiana* isolates were subjected to genetic analysis using a GBS approach. Of these, 180 isolates generated sufficient GBS reads (ranging from 1,022,123 to 7,778,526 reads, with an average of 4,048,201 reads) and were considered for further data processing. The unfiltered VCF file resulting from alignment of GBS reads to an in-house *C. monteithiana* reference genome contained 179,118 SNPs across the 180 isolates. Subsequent filtering for minor allele frequency, biallelic sites, read depth, and missing data resulted in a dataset comprised of 11,477 SNPs across 149 *C. monteithiana* isolates (Table S2.2). Heterozygous sites were removed but accounted for 2.87% of all sites prior to filtering, with the proportion of heterozygous SNPs in individuals ranging from 0.36% to 7.28% (Figure S2.2). The 149 *C. monteithiana* isolates included in the dataset used for downstream genetic structure and diversity analyses were acquired from nine grass hosts and 112 different Georgia counties (Figure 2.1 and Table S2.1).

**Population structure of *C. monteithiana***

In determining the optimal number of populations, the delta K graph generated from STRUCTURE runs showed a clear peak at K = 2 (Figure 2.2). Therefore, two populations were identified, with population 1 (Pop 1) consisting of 123 *C. monteithiana* isolates and population 2 (Pop 2) consisting of 26. Moreover, the phylogenetic analysis also revealed two distinct populations (Figure 2.2), and the PCoA further corroborated this result, with principal components 1 and 2 representing 25.32% and 7.17% of the genetic variability within the dataset, respectively (Figure 2.3). No admixed individuals were identified. Furthermore, based on Mantel tests, no significant correlation between geographic and genetic distances was detected among all

isolates ($P$ = 0.466, Rxy = 0.002) or among isolates within each population (Pop 1 $P$ = 0.143, Rxy = 0.044; Pop 2 $P$ = 0.054, Rxy = 0.111). Similarly, no apparent clustering was observed based on turfgrass host or by the year in which isolates were collected (Figure 2.3). Lastly, no population-specific SNPs (i.e. private SNPs) were identified in the sequenced ITS region.

**Genetic diversity of *C. monteithiana***

Genetic diversity indices categorized by population are presented in Table 2.1. Across all samples, each *C. monteithiana* isolate was found to represent a unique MLG (n = 149), and the genotype accumulation curve showed that only a small proportion (~350 SNPs) of the total number of SNP markers was required to distinguish all MLGs (Figure S2.3). Therefore, the number of MLGs in each population directly corresponded to the number of individuals (Pop 1 MLG = 123; Pop 2 MLG = 26). Moreover, population 1 and population 2 exhibited similar levels of diversity as indicated by eMLG (eMLG = 26 and eMLG = 26, respectively), Shannon's information index (I = 0.251 and I = 0.201, respectively), and unbiased diversity (uh = 0.151 and uh = 0.127, respectively). Likewise, each population had a comparable number of effective alleles (Pop 1 Ne = 1.221; Pop 2 Ne = 1.179). The percentage of polymorphic loci was higher in population 1 (%P = 82.5) compared to population 2 (%P = 55.1), as was the number of private alleles (Pop 1 Pa = 5,410; Pop 2 Pa = 3,809), likely due to the higher number of samples in population 1 (> fourfold) compared to population 2. Gene flow between the two populations was low ($Nm$ = 0.542), whereas the coefficient of genetic differentiation was high ($G_{ST}$ = 0.481). Furthermore, tests for the index of association and the standardized index of association across the two populations rejected the null hypotheses of random mating (Pop 1 $I_A$ = 189, $r\bar{d}$ = 0.0382, $P$ = 0.001; Pop 2 $I_A$ = 113, $r\bar{d}$ = 0.0280, $P$ = 0.001) (Figure S2.4). Finally, the neighbor-net

phylogenetic analysis identified a few regions with conflicting phylogenetic signals, with reticulations appearing more frequently in population 1 than in population 2 (Figure S2.5).

**Discussion**

In this study, we investigated the genetic diversity and population structure of *C. monteithiana* sampled from various turfgrass hosts and locations across the state of Georgia using a GBS approach. Previous studies akin to ours that assess diversity of dollar spot pathogens have mostly done so using VCG assays (Deng et al., 2002; Powell and Vargas Jr., 2001; Viji et al., 2004). However, these assays involve a certain degree of imprecision and subjectivity, as they rely on macroscopic assessments of barrage formation between isolates to categorize them into compatibility groups (Chang et al., 2014). Furthermore, while older molecular marker technologies, such as RAPDs, ISSRs, and AFLPs, have been used to assess dollar spot pathogen diversity, our study is the first to use SNP markers generated from a high-throughput sequencing technique (GBS) for this purpose. SNP markers are preferred in modern population genetic research due to their biallelic and co-dominant nature, informative power, stability, scalability, and abundance across the genomes of several organisms (Helyar et al., 2011; Mammadov et al., 2012; Young and Vivier, 2010). Other studies that have utilized SNP markers in dollar spot pathogen research have mostly done so to identify new *Clarireedia* species by conducting multilocus sequencing analyses for a few housekeeping loci (e.g. ITS region, *CaM*, *Mcm7*, and *EF-1α* genes) (Hu et al., 2019; Salgado-Salazar et al., 2018; Zhang et al., 2022). The GBS approach in our work, however, provided a more comprehensive dataset for exploring diversity and structure by generating reduced-representation libraries of the whole genome, resulting in higher coverage and marker resolution/density.

Our initial objective in this study was to identify the predominant *Clarireedia* spp. causing dollar spot in Georgia. We hypothesized this species to be *C. monteithiana*, as most of our dollar spot isolates were gathered from warm-season turfgrasses, the most widely grown turfgrasses across the state. Identification of dollar spot samples through sequencing of the ITS region revealed that 96% of our isolates were indeed *C. monteithiana*. The high frequency of *C. monteithiana* recovery from warm-season turfgrasses aligns with the widely held notion that *Clarireedia* spp. exhibit host specificity (Salgado-Salazar et al., 2018). However, three of our *C. monteithiana* isolates were obtained from cool-season turfgrasses, and four out of eight of our *C. jacksonii* isolates were obtained from warm-season turfgrasses. This evidence opposing absolute host specificity has been corroborated by a few other studies, particularly through artificial cross inoculation experiments. By challenging both cool- and warm-season turfgrass hosts with *C. monteithiana* and *C. jacksonii* isolates, Sapkota et al. (2020) and Aynardi et al. (2019) found that both species were able to incite disease on both host types. Additionally, Aynardi et al. (2019) also noted that *C. jacksonii* was more virulent on both host types than *C. monteithiana* across all of their experiments, underscoring the potential importance of species identification in dollar spot management. Moreover, in their study describing a new dollar spot pathogen species, Hu et al. (2019) pointed out that *C. jacksonii* isolates in their collection were recovered from both cool- and warm-season hosts in China, affirming the occurrence of cross infection in nature. These exceptions to host specificity are more likely to occur in transition zones, such as parts of Georgia, where both cool- and warm-season turfgrass species are grown concurrently (Aynardi et al., 2019). To draw further conclusions on the dynamics of *Clarireedia* spp. host specificity in Georgia, a more expansive collection of dollar spot isolates from cool-season turfgrass hosts is likely needed. Nonetheless, our results suggest that *C. monteithiana* is the primary causal agent

of dollar spot on warm-season turfgrasses in Georgia and the most prevalent *Clarireedia* species causing dollar spot in the state.

Furthermore, while previous studies have uncovered some genetic variability within *Clarireedia* species through multilocus sequencing analyses (Aynardi et al., 2019; Hu et al., 2019; Salgado-Salazar et al., 2018; Zhang et al., 2022), our GBS data revealed diversity within *C. monteithiana* through the detection of two genetically distinct populations in Georgia. This is the first time population structure has been observed within *C. monteithiana*. In relation to our findings, two other studies have documented appreciable levels of genetic diversity among warm-season dollar spot isolate collections (presumably *C. monteithiana* isolates). The first was a VCG assay conducted by Sonoda (1989) using 119 dollar spot isolates obtained from *Paspalum notatum* (bahiagrass). The authors identified 54 different VCGs, by far the highest number of VCGs recorded for any dollar spot pathogen assay of this type. Additionally, all isolates used in their study came from a single state (Florida), echoing our findings that warm-season pathogen diversity can be observed at a smaller geographic scale (Sonoda, 1989). Similarly, Liberti et al. (2012) used a comparable approach to assess diversity of 47 isolates collected from mostly warm-season hosts in Florida (n = 29) (presumably *C. monteithiana*) and from cool-season hosts in four northern U.S. states (n = 18) (presumably *C. jacksonii*). The Florida isolates exhibited greater VCG diversity, as 14 of the 18 total VCGs (78%) identified were found exclusively in Florida. Moreover, the group also found ITS sequence diversity among their warm-season isolates in the form of an intron located at the 3' end of the SSU rDNA region. This intronic region was present in nearly 30% of their warm-season isolates and completely absent in cool-season isolates (Liberti et al., 2012). Likewise, a few years prior to this finding, Marek et al. (2008) also identified the same intron in two of their three dollar spot

isolates collected from a warm-season turfgrass host (*Buchloe dactyloides*, buffalograss) in Oklahoma. These observations align with results from our study, as we detected the same intronic region in 50 of our *C. monteithiana* isolates but not in *C. jacksonii*. The intron was identified in isolates from various locations (counties) and hosts and was present in both populations, suggesting that its insertion into the SSU region occurred prior to population divergence. To our knowledge, this specific ITS sequence polymorphism has not been observed in cool-season dollar spot isolates, and our study, along with Liberti et al. (2012) and Marek et al. (2008), are the only ones to document its occurrence within the continental U.S. However, a similar intron (78% sequence similarity) in the ITS region has been identified in *C. paspali* isolates recovered from seashore paspalum in China (Hu et al., 2019).

While two distinct *C. monteithiana* populations were identified in Georgia, we found that the turfgrass species from which isolates were derived did not affect population structure, nor did the year or location from which isolates were sampled. In light of this, we hypothesized that fungicide sensitivity might explain the population structure observed in our study, as it has proven to be an important driver of structure in other pathosystems, such as *Botrytis* spp. on berry crops (Naegele et al., 2022), *Phytophthora capsici* on vegetable crops (Parada-Rojas and Quesada-Ocampo, 2022), and *Gaeumannomyces graminis* var. *tritici* on wheat (Freeman et al., 2005). However, in comparing our work with results from Ghimire et al. (2023), who included a subset of 75 *C. monteithiana* isolates from our collection in fungicide sensitivity assays (benzimidazoles and dimethyl inhibitor fungicides), we did not find a meaningful association between population structure and fungicide sensitivity (data not shown). Nevertheless, the effect of fungicides on dollar spot pathogen populations warrants ongoing consideration, as frequent applications often used for dollar spot control could impose selection pressures that facilitate

resistance development and subsequently alter population dynamics over time (Huzar-Novakowiski and Dorrance, 2018; Latin, 2006; Stephens and Kaminski, 2019). Furthermore, other possible factors unaccounted for in our study that could have influenced *C. monteithiana* population structure include pathogen aggressiveness and virulence spectrum. Variations in these traits are often shaped by adaptation to local climatic conditions (e.g. temperature, moisture, nutrient availability), host genetic background, and host resistance mechanisms (Pariaud et al., 2009; Tack et al., 2012). Previous greenhouse and field studies have demonstrated variability in dollar spot severity (i.e. aggressiveness) and virulence patterns in seashore paspalum (Benda et al., 2017; Steketee et al., 2016) and creeping bentgrass (Chakraborty et al., 2006). Moreover, variations in these traits have shaped or correlated with the population structure of several plant pathogens, including *Puccinia striiformis* f. sp. *tritici* (Milus et al., 2009), *Fusarium graminearum* (Zhang et al., 2012), and *Ascochyta rabiei* (Bar et al., 2021). Evaluating the aggressiveness and virulence spectrum among our *C. monteithiana* isolates through pathogenicity tests on a diverse host panel may reveal how or if they affect population structure. This in turn could have direct implications for dollar spot management in Georgia.

In characterizing *C. monteithiana* at the population level, population 1 was much larger than population 2 (n = 123, n = 26, respectively). However, both populations exhibited similar levels of genetic diversity, which may indicate a shared ancestral origin in this region of the country. Alternatively, the emergence of these two populations could be explained by migratory events, either through a single introduction or two separate introductions that occurred close together in time. The frequent transportation of turfgrasses, particularly warm-season turfgrasses, throughout the southeastern United States is one possibility that might explain these introduction events. Many popular warm-season turfgrass varieties are vegetatively propagated through

sodding or sprigging, rather than seeding (Hanna et al., 2013). Therefore, a living stock of these varieties must be continuously grown and maintained for turfgrass practitioners to utilize them, and this is typically done at sod farms. Sod farms ship source plant material to various long- or short-distance locations as needed, and plant pathogens, including *C. monteithiana*, are inevitably shipped along with it. Thus, the unintentional spread of pathogens, particularly different pathogen strains, through transported sod could explain the presence of two distinct *C. monteithiana* populations in Georgia.

Furthermore, index of association and standardized index of association tests for each population strongly rejected the null hypothesis for random mating, supporting the clonal reproduction of *C. monteithiana*. The indication of clonal populations is consistent with several previous observations pertaining to dollar spot pathogen biology (DeVries et al., 2008), particularly those citing lack of sexual or asexual spore production (Espevig et al., 2017; Putman et al., 2015; Salgado-Salazar et al., 2018). Considering this, along with the observation of limited gene flow between populations ($Nm = 0.542$), we infer that mutation is the primary driver of genetic variation within *C. monteithiana* in Georgia. However, conflicting phylogenetic signals detected in neighbor-net analysis suggest that recombination could still be a contributing factor to the pathogen's diversity. Along these lines, heterokaryosis or parasexuality may influence diversity to some extent, as heterozygous sites represented 2.87% of all sites in our GBS dataset, with individual heterozygosity levels ranging from 0.36% to 7.28%. These processes have been shown to contribute to the genetic variability of a few clonal plant pathogens, including *Cryphonectria parasitica* (Milgroom et al., 2009), *Alternaria alternata* (Stewart et al., 2013), and *Magnaporthe grisea* (Zeigler et al., 1997), and might play a similar role in *Clarireeidia* (Hulvey et al., 2012; Jo et al., 2008b; Kessler et al., 2018; Liberti et al., 2012).

## Conclusion

Despite the prevalence of dollar spot in turfgrasses throughout the southeastern United States, little is known about the population genetics of the causal pathogens in this region. The present study aimed to fill these knowledge gaps by exploring the genetic diversity and population structure of *C. monteithiana* in Georgia, using a GBS approach. Our findings uncovered that *C. monteithiana* was indeed the most prevalent *Clarireedia* species in the state, and pointed toward the presence of two clonal *C. monteithiana* populations. Although the underlying factors contributing to divergence of these populations were unclear, our results provide a baseline for future research to address this question. In terms of management, diverse pathogen populations have higher evolutionary potential to overcome control strategies such as fungicide applications and host resistance. Our research revealed diversity within *C. monteithiana*, as does other research related to dollar spot pathogens of warm-season turfgrasses. Therefore, future efforts to assess and monitor *C. monteithiana* genetic diversity and structure will be beneficial in preventing failure or reduced efficacy of current dollar spot management tools.

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**Table 2.1:** Genetic diversity indices generated using the *poppr* package in R v4.3.2 and GenAlEx v6.51b2 for the two *C. monteithiana* populations based on 11,477 GBS markers, derived from a collection of 149 *C. monteithiana* isolates sampled in Georgia turfgrasses from 2019 to 2023.

| Population | NI[a] | MLG[b] | eMLG[c] | %P[d] | I[e] | uh[f] | Ne[g] | Pa[h] | $I_A$[i] | $r\bar{d}$[j] | $Nm$[k] | $G_{ST}$[l] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pop 1 | 123 | 123 | 26 | 82.5 | 0.251 | 0.151 | 1.221 | 5,410 (0.008-0.992) | 189 | 0.0382 | 0.542 | 0.481 |
| Pop 2 | 26 | 26 | 26 | 55.1 | 0.201 | 0.127 | 1.179 | 3,809 (0.038-0.962) | 113 | 0.0280 | | |
| Total/Avg. | 149 | 149 | 26 | 68.8 | 0.226 | 0.139 | 1.200 | 9,219 | 753 | 0.0906 | | |

[a] NI = number of isolates
[b] MLG = number of multilocus genotypes
[c] eMLG = expected number of multilocus genotypes based on rarefaction analysis
[d] %P = percentage of polymorphic loci
[e] I = Shannon's information index
[f] uh = unbiased diversity
[g] Ne = number of effective alleles
[h] Pa = number of private alleles (frequency range)
[i] $I_A$ = index of association
[j] $r\bar{d}$ = standardized index of association
[k] $Nm$ = estimate of gene flow
[l] $G_{ST}$ = coefficient of genetic differentiation

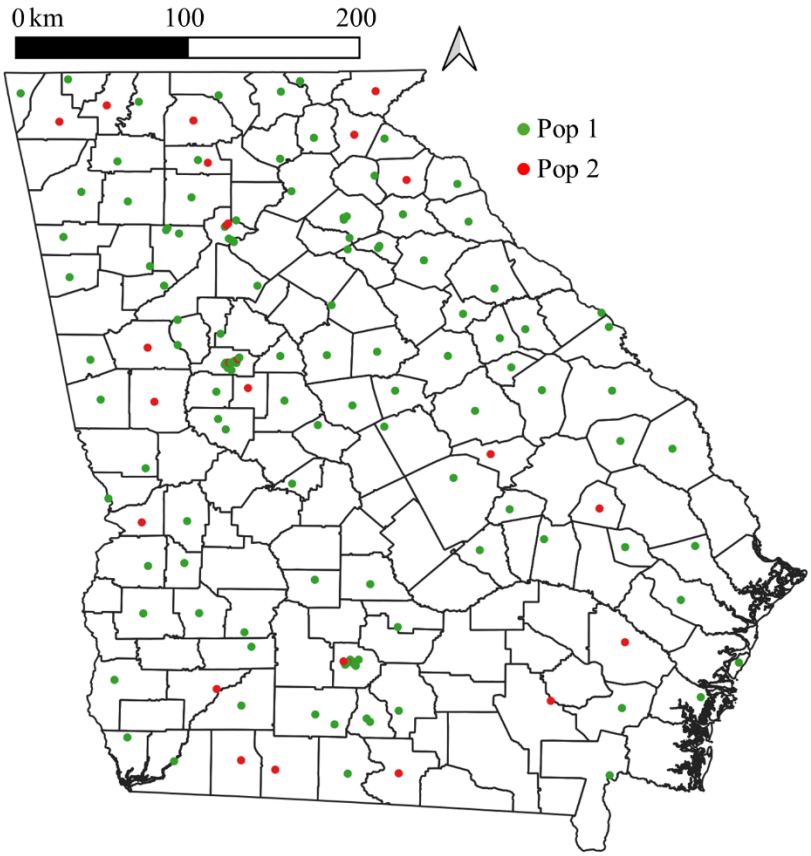


**Figure 2.1:** Map of Georgia (created in QGIS v3.34.9) showing sampling locations of 149 *C. monteithiana* isolates collected from turfgrass across 112 counties from 2019 to 2023 and used for population structure and genetic diversity analyses. Isolates are color-coded by population membership (Pop 1, green; Pop 2, red), according to STRUCTURE results at K=2.

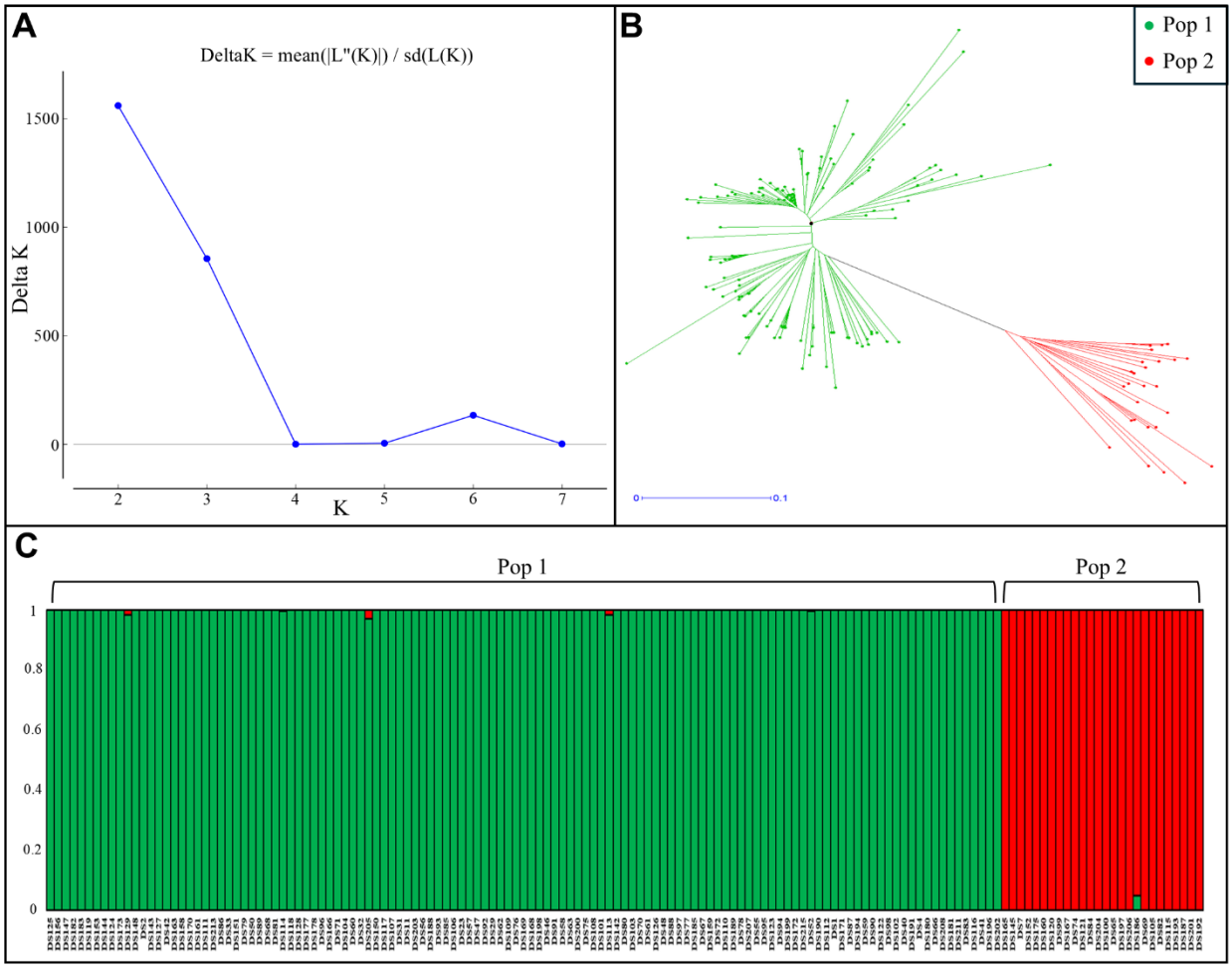


**Figure 2.2:** Population structure of 149 *C. monteithiana* isolates sampled in Georgia turfgrasses from 2019 to 2023, based on 11,477 GBS markers. **A**, Delta K graph showing the change in likelihood for different numbers of inferred genetic groupings, with a sharp peak at K = 2. **B**, Neighbor-joining phylogenetic tree, based on a dissimilarity matrix, showing isolates separating into two populations. **C**, STRUCTURE bar plot illustrating assignment of individuals to the different populations (Pop 1, green; Pop 2, red).

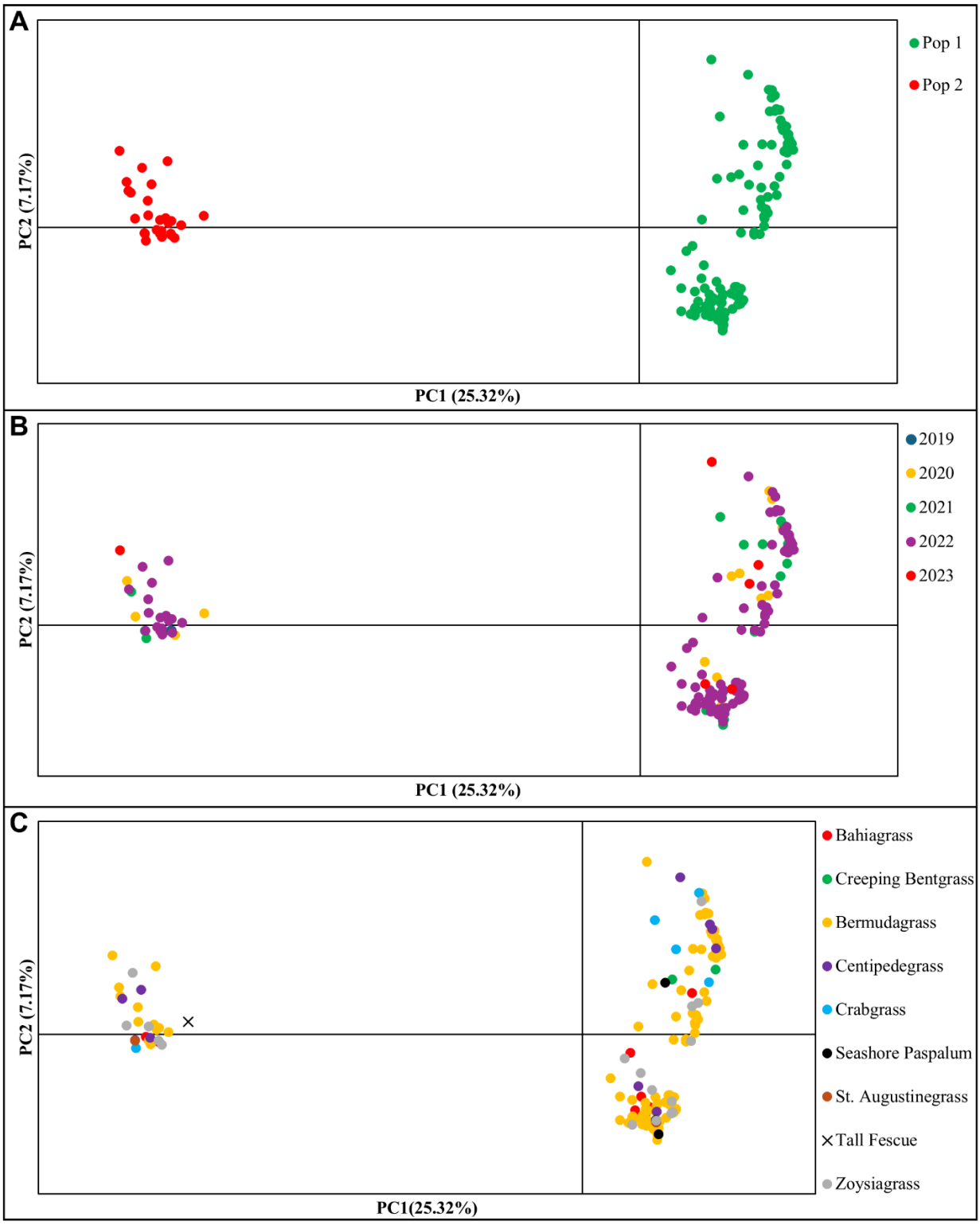


**Figure 2.3:** Principal coordinate analysis (PCoA) for 149 *C. monteithiana* isolates sampled in Georgia turfgrasses from 2019 to 2023, based on 11,477 GBS markers. **A**, PCoA plot depicting the genetic differentiation between individuals in two distinct populations, supporting the results from STRUCTURE analysis. **B** and **C**, Corresponding PCoA plots categorizing isolates by year of collection and by turfgrass host, respectively.

## Supplemental Materials

**Table S2.1:** Details on all *Clarireedia* isolates sampled in Georgia turfgrasses from 2019 to 2023 (n = 210).

| Isolate ID* | *Clarireedia* species | Host | Year | Season | County | Site Type | Number of GBS Reads | Population | Presence of SSU intron |
|---|---|---|---|---|---|---|---|---|---|
| DS1* | *C. monteithiana* | Zoysiagrass | 2020 | Fall | Clarke | Residential | 4212335 | Pop 1 | Yes |
| DS2* | *C. monteithiana* | Zoysiagrass | 2020 | Fall | Bibb | Residential | 3622807 | Pop 1 | No |
| DS3 | *C. jacksonii* | Creeping Bentgrass | 2019 | Fall | Spalding | University | - | - | No |
| DS4* | *C. monteithiana* | Zoysiagrass | 2020 | Fall | Spalding | Landscape | 3676975 | Pop 1 | No |
| DS7* | *C. monteithiana* | Zoysiagrass | 2019 | Fall | Spalding | University | 3883743 | Pop 2 | Yes |
| DS8 | *C. monteithiana* | Seashore Paspalum | 2019 | Fall | Spalding | University | 4090938 | - | No |
| DS9 | *C. monteithiana* | Bermudagrass | 2019 | Fall | Spalding | University | 3628047 | - | No |
| DS10 | *C. monteithiana* | Zoysiagrass | 2021 | Spring | Spalding | University | 3575700 | - | No |
| DS11* | *C. monteithiana* | Bermudagrass | 2021 | Spring | Spalding | University | 3600420 | Pop 1 | No |
| DS12 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Fulton | Golf Course | 0 | - | No |
| DS13 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Lincolnton | Golf Course | 0 | - | No |
| DS14 | *C. monteithiana* | Zoysiagrass | 2021 | Summer | Clarke | Residential | 0 | - | No |
| DS15 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Fayette | Landscape | 0 | - | No |
| DS16 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Fayette | Landscape | 0 | - | Yes |
| DS17 | *C. monteithiana* | Carpetgrass | 2021 | Summer | Fayette | Residential | 0 | - | No |
| DS18 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Fayette | Landscape | 0 | - | No |
| DS19 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Fayette | Landscape | 0 | - | No |
| DS20 | *C. monteithiana* | Zoysiagrass | 2021 | Summer | Coweta | Residential | 0 | - | No |
| DS21 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Columbia | Landscape | 0 | - | No |
| DS22 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Greene | Landscape | 0 | - | Yes |
| DS23* | *C. monteithiana* | Bermudagrass | 2021 | Summer | Morgan | Landscape | 6185009 | Pop 1 | Yes |
| DS24 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Spalding | Landscape | 6106303 | - | Yes |
| DS25 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Rockdale | Landscape | 6856927 | - | No |
| DS26 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Henry | Landscape | 6523156 | - | Yes |
| DS27 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Newton | Landscape | 7528796 | - | No |
| DS28 | *C. monteithiana* | Zoysiagrass | 2021 | Summer | Harris | Residential | 6795382 | - | No |
| DS29* | *C. monteithiana* | Bermudagrass | 2021 | Summer | Harris | Residential | 6975675 | Pop 1 | No |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DS30 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Gwinnett | Residential | 6020116 | - | No |
| DS31* | *C. monteithiana* | Bermudagrass | 2021 | Summer | DeKalb | Landscape | 7609905 | Pop 1 | No |
| DS32* | *C. monteithiana* | Bermudagrass | 2021 | Summer | Cobb | Residential | 7072620 | Pop 1 | Yes |
| DS33* | *C. monteithiana* | Bermudagrass | 2021 | Summer | Cobb | Residential | 7778526 | Pop 1 | No |
| DS34 | *C. monteithiana* | Zoysiagrass | 2021 | Summer | Fayette | Landscape | 7626786 | - | No |
| DS35 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Walton | Landscape | 6485626 | - | Yes |
| DS36 | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | Landscape | 6811384 | - | Yes |
| DS37 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Tift | Landscape | 6157782 | - | No |
| DS38 | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | Landscape | 6833404 | - | Yes |
| DS39 | *C. monteithiana* | Bermudagrass | 2021 | Summer | Tift | Landscape | 5481419 | - | Yes |
| DS40* | *C. monteithiana* | Seashore Paspalum | 2021 | Fall | Cook | Sod Farm | 6625295 | Pop 1 | No |
| DS41* | *C. monteithiana* | Creeping Bentgrass | 2021 | Fall | Fayette | Golf Course | 7551273 | Pop 1 | No |
| DS42* | *C. monteithiana* | Bermudagrass | 2021 | Fall | Fayette | Golf Course | 5703292 | Pop 1 | No |
| DS46* | *C. monteithiana* | Bermudagrass | 2022 | Spring | Fulton | Golf Course | 3606586 | Pop 1 | No |
| DS47* | *C. monteithiana* | Bermudagrass | 2022 | Spring | Fulton | Golf Course | 3979875 | Pop 1 | No |
| DS48* | *C. monteithiana* | Bermudagrass | 2022 | Spring | Fulton | Golf Course | 4060541 | Pop 1 | No |
| DS49 | *C. jacksonii* | Bermudagrass | 2022 | Spring | Carroll | Athletic Field | - | - | No |
| DS50* | *C. monteithiana* | Bermudagrass | 2022 | Spring | Clarke | Landscape | 4145999 | Pop 1 | No |
| DS51 | *C. monteithiana* | Bermudagrass | 2022 | Summer | Spalding | University | 1022123 | - | No |
| DS52* | *C. monteithiana* | Bahiagrass | 2022 | Summer | Spalding | University | 4426370 | Pop 1 | No |
| DS53 | *C. jacksonii* | Bermudagrass | 2022 | Summer | Spalding | University | - | - | No |
| DS54 | *C. jacksonii* | St. Augustinegrass | 2022 | Summer | Spalding | University | - | - | No |
| DS55* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Spalding | University | 4357404 | Pop 1 | Yes |
| DS56* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Columbia | Landscape | 3960929 | Pop 1 | No |
| DS57* | *C. monteithiana* | Zoysiagrass | 2022 | Summer | Richmond | Residential | 3634553 | Pop 1 | Yes |
| DS58* | *C. monteithiana* | Bahiagrass | 2022 | Spring | McIntosh | Landscape | 3809107 | Pop 1 | Yes |
| DS59* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Jackson | Landscape | 4381965 | Pop 1 | No |
| DS60* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Jackson | Athletic Field | 3861651 | Pop 1 | No |
| DS61* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Jackson | Landscape | 3986184 | Pop 1 | No |
| DS62* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Fayette | Landscape | 4522955 | Pop 1 | No |
| DS63* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Cobb | Landscape | 3789537 | Pop 1 | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DS64 | *C. monteithiana* | Bermudagrass | 2022 | Summer | Dawson | Residential | 3825279 | - | No |
| DS65* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Whitfield | Landscape | 4090979 | Pop 2 | No |
| DS66* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Jasper | Landscape | 4772937 | Pop 1 | No |
| DS67* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Cherokee | Landscape | 4856259 | Pop 1 | No |
| DS68* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Fannin | Landscape | 5272615 | Pop 1 | No |
| DS69* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Gilmer | Landscape | 4476373 | Pop 2 | No |
| DS70* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Murray | Landscape | 3654036 | Pop 1 | No |
| DS71* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Catoosa | Landscape | 4009647 | Pop 1 | No |
| DS72* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Dade | Landscape | 2891144 | Pop 1 | No |
| DS73 | *C. monteithiana* | Bermudagrass | 2022 | Summer | Chattooga | Athletic Field | 4037682 | - | No |
| DS74* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Walker | Athletic Field | 3767039 | Pop 2 | No |
| DS75* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Gordon | Landscape | 3658153 | Pop 1 | No |
| DS76* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Bartow | Landscape | 3883342 | Pop 1 | No |
| DS77* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Floyd | Landscape | 3988926 | Pop 1 | Yes |
| DS78* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Douglas | Landscape | 4014079 | Pop 1 | No |
| DS79* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Polk | Landscape | 3427779 | Pop 1 | No |
| DS80* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Paudling | Athletic Field | 4619821 | Pop 1 | No |
| DS81* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Haralson | Landscape | 3511005 | Pop 1 | No |
| DS82* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Pickens | Landscape | 3830544 | Pop 2 | No |
| DS83* | *C. monteithiana* | Zoysiagrass | 2022 | Summer | Spalding | University | 4007911 | Pop 1 | Yes |
| DS84* | *C. monteithiana* | St. Augustinegrass | 2022 | Summer | Lamar | Landscape | 4729025 | Pop 2 | No |
| DS85* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Hancock | Athletic Field | 3458232 | Pop 1 | Yes |
| DS86* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Oconee | Residential | 2585087 | Pop 1 | No |
| DS87* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Taliaferro | Landscape | 3716330 | Pop 1 | No |
| DS88* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Jasper | Landscape | 3735319 | Pop 1 | No |
| DS89* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Butts | Landscape | 3620635 | Pop 1 | No |
| DS90* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Barrow | Landscape | 3458836 | Pop 1 | Yes |
| DS91* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Putnam | Landscape | 3225607 | Pop 1 | Yes |
| DS92* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Oglethorpe | Landscape | 3890861 | Pop 1 | Yes |
| DS93* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Hall | Landscape | 3264840 | Pop 1 | No |
| DS94* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Union | Landscape | 4090675 | Pop 1 | No |

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|---|---|---|---|---|---|---|---|---|---|
| DS95* | *C. monteithiana* | Bermudagrass | 2022 | Summer | White | Landscape | 4016263 | Pop 1 | Yes |
| DS96* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Stephens | Landscape | 3968717 | Pop 1 | No |
| DS97* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Lumpkin | Landscape | 3261007 | Pop 1 | No |
| DS98* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Clayton | Landscape | 3402656 | Pop 1 | Yes |
| DS99* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Rabun | Landscape | 4541621 | Pop 2 | No |
| DS100* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Franklin | Landscape | 3827562 | Pop 2 | No |
| DS101* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Forsyth | Landscape | 4427482 | Pop 1 | No |
| DS102* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Towns | Landscape | 3600849 | Pop 1 | Yes |
| DS103* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Banks | Landscape | 3424456 | Pop 1 | No |
| DS104* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Madison | Landscape | 3598581 | Pop 1 | Yes |
| DS105* | *C. monteithiana* | Zoysiagrass | 2022 | Summer | Habersham | Landscape | 3718516 | Pop 2 | Yes |
| DS106* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Wilkes | Golf Course | 3895159 | Pop 1 | No |
| DS107* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Jefferson | Athletic Field | 3956700 | Pop 1 | No |
| DS108* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Warren | Landscape | 2808459 | Pop 1 | No |
| DS109* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Glascock | Landscape | 3138650 | Pop 1 | No |
| DS110* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Hart | Landscape | 4738501 | Pop 1 | No |
| DS111* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Burke | Landscape | 3751278 | Pop 1 | No |
| DS112* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Elbert | Landscape | 4212513 | Pop 1 | No |
| DS113* | *C. monteithiana* | Bermudagrass | 2022 | Summer | McDuffie | Landscape | 3733537 | Pop 1 | No |
| DS114* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Washington | Landscape | 4833447 | Pop 1 | Yes |
| DS115* | *C. monteithiana* | Bahiagrass | 2022 | Summer | Lowndes | Landscape | 3207778 | Pop 2 | No |
| DS116* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Jones | Landscape | 3948483 | Pop 1 | Yes |
| DS117* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Upson | Landscape | 4803801 | Pop 1 | Yes |
| DS118* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Monroe | Landscape | 4451364 | Pop 1 | Yes |
| DS119* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Pike | Landscape | 2654239 | Pop 1 | Yes |
| DS120* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Meriweather | Landscape | 4665420 | Pop 2 | No |
| DS121* | *C. monteithiana* | Centipedegrass | 2022 | Summer | Ware | Residential | 3905046 | Pop 2 | No |
| DS122* | *C. monteithiana* | Zoysiagrass | 2022 | Summer | Troup | Landscape | 3920116 | Pop 1 | No |
| DS123* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Heard | Landscape | 3915741 | Pop 1 | No |
| DS124* | *C. monteithiana* | Bermudagrass | 2022 | Summer | Baldwin | Athletic Field | 4225628 | Pop 1 | No |
| DS125* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Peach | Landscape | 4774753 | Pop 1 | No |

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|---|---|---|---|---|---|---|---|---|---|
| DS126* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Lee | Landscape | 4240650 | Pop 1 | No |
| DS127* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Terrel | Landscape | 4254430 | Pop 1 | No |
| DS128* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Dougherty | Landscape | 3608446 | Pop 1 | No |
| DS129* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Webster | Landscape | 3764411 | Pop 1 | No |
| DS130 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Macon | Landscape | 4026367 | - | Yes |
| DS131 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Schley | Landscape | 0 | - | Yes |
| DS132 | *C. monteithiana* | Zoysiagrass | 2022 | Fall | Talbot | Landscape | 0 | - | No |
| DS133 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Houston | Landscape | 0 | - | No |
| DS134 | *C. monteithiana* | Centipedegrass | 2022 | Fall | Terrel | Landscape | 0 | - | No |
| DS135 | *C. monteithiana* | Bahiagrass | 2022 | Fall | Quitman | Landscape | 0 | - | No |
| DS136 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Calhoun | Landscape | 0 | - | No |
| DS137 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Worth | Landscape | 0 | - | No |
| DS138 | *C. monteithiana* | Zoysiagrass | 2022 | Fall | Sumter | Landscape | 0 | - | No |
| DS139 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Dooly | Landscape | 0 | - | No |
| DS140 | *C. monteithiana* | Zoysiagrass | 2022 | Fall | Taylor | Landscape | 0 | - | Yes |
| DS141 | *C. monteithiana* | St. Augustinegrass | 2022 | Fall | Crawford | Landscape | 0 | - | No |
| DS142* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Crisp | Athletic Field | 3474591 | Pop 1 | No |
| DS143* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Stewart | Landscape | 4074865 | Pop 1 | No |
| DS144* | *C. monteithiana* | Zoysiagrass | 2022 | Fall | Marion | Landscape | 3422828 | Pop 1 | No |
| DS145* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Chattahoochee | Athletic Field | 3729997 | Pop 2 | No |
| DS146 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Turner | Landscape | 3669576 | - | Yes |
| DS147* | *C. monteithiana* | Zoysiagrass | 2022 | Fall | Randolph | Landscape | 3195291 | Pop 1 | Yes |
| DS148* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Muscogee | Landscape | 3611881 | Pop 1 | No |
| DS149 | *C. monteithiana* | Centipedegrass | 2022 | Fall | Montgomery | Landscape | 4016378 | - | No |
| DS150* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Laurens | Athletic Field | 4155650 | Pop 1 | No |
| DS151* | *C. monteithiana* | Centipedegrass | 2022 | Fall | Wilkinson | Athletic Field | 3456115 | Pop 1 | Yes |
| DS152* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Candler | Landscape | 3429943 | Pop 2 | No |
| DS153* | *C. monteithiana* | Centipedegrass | 2022 | Fall | Toombs | Landscape | 2533217 | Pop 1 | Yes |
| DS154 | *C. monteithiana* | Centipedegrass | 2022 | Fall | Emanuel | Landscape | 2372671 | - | No |
| DS155 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Emanuel | Golf Course | 3436124 | - | Yes |
| DS156* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Jenkins | Athletic Field | 3064174 | Pop 1 | No |

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|---|---|---|---|---|---|---|---|---|---|
| DS157 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Bulloch | Athletic Field | 3855273 | - | No |
| DS158* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Treutlen | Athletic Field | 4196472 | Pop 1 | Yes |
| DS159* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Wheeler | Athletic Field | 3245740 | Pop 1 | No |
| DS160* | *C. monteithiana* | Zoysiagrass | 2022 | Fall | Johnson | Landscape | 3311758 | Pop 2 | Yes |
| DS161* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Screven | Landscape | 4060126 | Pop 1 | Yes |
| DS162 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Effingham | Athletic Field | 3799982 | - | No |
| DS163* | *C. monteithiana* | St. Augustinegrass | 2022 | Fall | Evans | Landscape | 3983338 | Pop 1 | Yes |
| DS164 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Tattnall | Landscape | 3434877 | - | No |
| DS165* | *C. monteithiana* | Bahiagrass | 2022 | Fall | Thomas | Landscape | 4056046 | Pop 2 | No |
| DS166* | *C. monteithiana* | Bahiagrass | 2022 | Fall | Ben Hill | Landscape | 3563772 | Pop 1 | No |
| DS167* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Grady | Athletic Field | 2886467 | Pop 2 | No |
| DS168* | *C. monteithiana* | Bahiagrass | 2022 | Fall | Brooks | Landscape | 2834471 | Pop 1 | Yes |
| DS169* | *C. monteithiana* | Bahiagrass | 2022 | Fall | Decatur | Landscape | 3129885 | Pop 1 | No |
| DS170* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Wilcox | Landscape | 3350617 | Pop 1 | Yes |
| DS171* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Early | Athletic Field | 3611937 | Pop 1 | No |
| DS172* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Seminole | Athletic Field | 3430308 | Pop 1 | No |
| DS173* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Colquitt | Landscape | 3913783 | Pop 1 | Yes |
| DS174 | *C. monteithiana* | Bermudagrass | 2022 | Fall | Miller | Landscape | 3526487 | - | No |
| DS175* | *C. monteithiana* | Centipedegrass | 2022 | Fall | Baker | Landscape | 3132251 | Pop 2 | No |
| DS176 | *C. monteithiana* | St. Augustinegrass | 2022 | Fall | Irwin | Landscape | 3819309 | - | No |
| DS177* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Berrien | Athletic Field | 3734193 | Pop 1 | No |
| DS178* | *C. monteithiana* | Bermudagrass | 2022 | Fall | Mitchell | Athletic Field | 3420175 | Pop 1 | No |
| DS179 | *C. monteithiana* | Zoysiagrass | 2020 | Fall | Clarke | Residential | 3607368 | - | No |
| DS180* | *C. monteithiana* | Zoysiagrass | 2020 | Summer | Spalding | University | 3534886 | Pop 1 | No |
| DS181* | *C. monteithiana* | Zoysiagrass | 2020 | Summer | Fulton | Residential | 3301661 | Pop 1 | No |
| DS182* | *C. monteithiana* | Bermudagrass | 2020 | Summer | Cook | Landscape | 2674652 | Pop 1 | No |
| DS183* | *C. monteithiana* | Seashore Paspalum | 2020 | Summer | Cook | Residential | 3272111 | Pop 1 | No |
| DS184 | *C. monteithiana* | Bermudagrass | 2020 | Summer | Spalding | University | 2994880 | - | Yes |
| DS185* | *C. monteithiana* | Crabgrass | 2020 | Summer | Spalding | Landscape | 3266324 | Pop 1 | No |
| DS186* | *C. monteithiana* | Tall Fescue | 2020 | Fall | Spalding | University | 3207117 | Pop 2 | No |
| DS187* | *C. monteithiana* | Zoysiagrass | 2020 | Summer | Fulton | Residential | 3080195 | Pop 2 | No |

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|---|---|---|---|---|---|---|---|---|---|
| DS188* | *C. monteithiana* | Creeping Bentgrass | 2020 | Fall | Spalding | University | 3936544 | Pop 1 | Yes |
| DS189* | *C. monteithiana* | Bermudagrass | 2020 | Summer | Spalding | Landscape | 3289403 | Pop 1 | No |
| DS190* | *C. monteithiana* | Seashore Paspalum | 2020 | Summer | Spalding | University | 4195960 | Pop 1 | No |
| DS191* | *C. monteithiana* | Zoysiagrass | 2020 | Summer | Spalding | Landscape | 3525882 | Pop 1 | No |
| DS192* | *C. monteithiana* | Bermudagrass | 2020 | Summer | Coweta | Residential | 3858590 | Pop 2 | No |
| DS193* | *C. monteithiana* | Zoysiagrass | 2020 | Fall | Fulton | Residential | 3019979 | Pop 2 | No |
| DS194* | *C. monteithiana* | Zoysiagrass | 2020 | Summer | Upson | Landscape | 3193439 | Pop 1 | No |
| DS195 | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | University | 3554343 | - | No |
| DS196* | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | University | 4479545 | Pop 1 | No |
| DS197* | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | University | 4427746 | Pop 2 | No |
| DS198* | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | University | 3614129 | Pop 1 | No |
| DS199* | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | Residential | 3171812 | Pop 1 | No |
| DS200* | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | Residential | 3329386 | Pop 1 | No |
| DS201* | *C. monteithiana* | Centipedegrass | 2021 | Summer | Tift | Residential | 3829606 | Pop 2 | No |
| DS202* | *C. monteithiana* | Crabgrass | 2021 | Summer | Tift | University | 3289259 | Pop 1 | Yes |
| DS203* | *C. monteithiana* | Crabgrass | 2021 | Summer | Tift | University | 3811564 | Pop 1 | No |
| DS204* | *C. monteithiana* | Crabgrass | 2021 | Summer | Tift | University | 2608804 | Pop 2 | No |
| DS205* | *C. monteithiana* | Crabgrass | 2021 | Summer | Tift | University | 2725393 | Pop 1 | No |
| DS206* | *C. monteithiana* | Bermudagrass | 2023 | Spring | Wayne | Athletic Field | 3563217 | Pop 2 | No |
| DS207* | *C. monteithiana* | Bermudagrass | 2023 | Spring | Brantley | Landscape | 3145156 | Pop 1 | No |
| DS208* | *C. monteithiana* | Bermudagrass | 2023 | Spring | Glynn | Athletic Field | 4277039 | Pop 1 | No |
| DS209 | *C. jacksonii* | Bermudagrass | 2023 | Spring | Long | Athletic Field | - | - | No |
| DS210 | *C. jacksonii* | Annual Ryegrass | 2023 | Spring | Pierce | Athletic Field | - | - | No |
| DS211* | *C. monteithiana* | Bermudagrass | 2023 | Spring | Bryan | Golf Course | 3161451 | Pop 1 | No |
| DS212 | *C. jacksonii* | Annual Ryegrass | 2023 | Spring | Camden | Athletic Field | - | - | No |
| DS213* | *C. monteithiana* | Bahiagrass | 2023 | Spring | Charlton | Landscape | 4083612 | Pop 1 | No |
| DS214 | *C. jacksonii* | Annual Ryegrass | 2023 | Spring | Chatham | Athletic Field | - | - | No |
| DS215* | *C. monteithiana* | Bahiagrass | 2023 | Spring | Liberty | Landscape | 3545628 | Pop 1 | No |

*Indicates *C. monteithiana* isolates retained for population structure and genetic diversity analyses following GBS data filtering (n = 149).

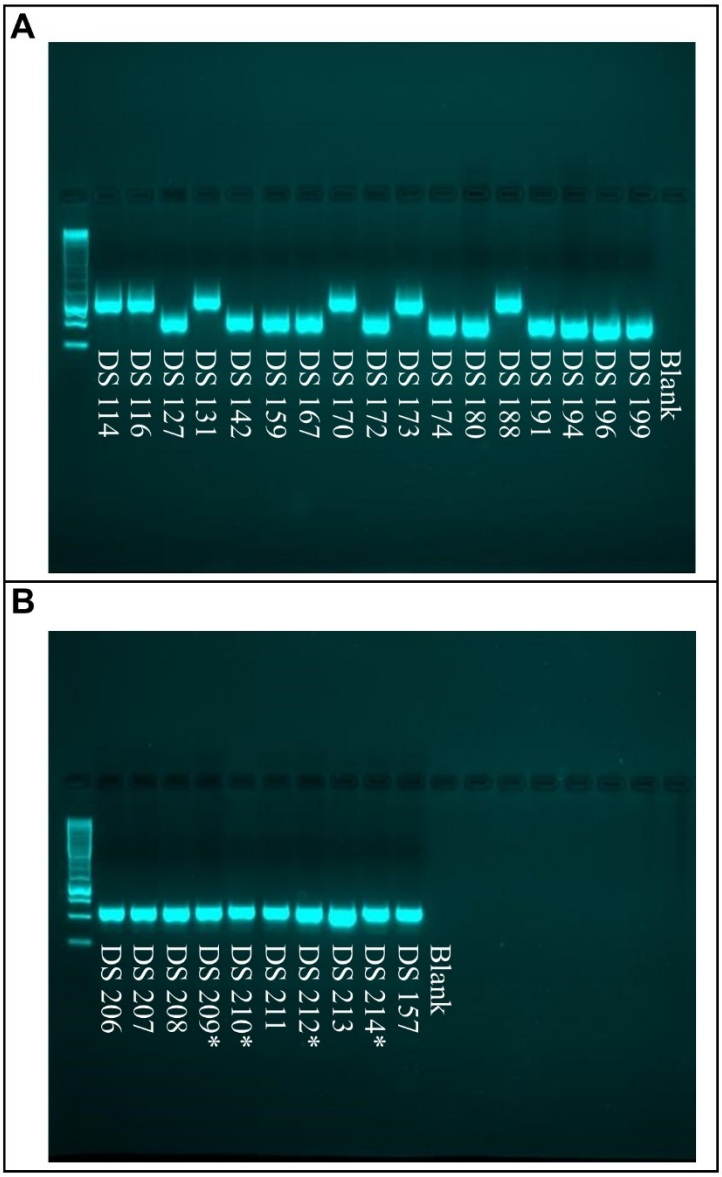


**Figure S2.1:** Agarose gel electrophoresis depicting PCR amplification of the ITS region for subsets of *Clarireedia* isolates. **A**, Amplification of only *C. monteithiana* isolates, six of which show a distinct banding pattern indicating the presence of the 3' end small subunit intron. **B**, Amplification of *C. monteithiana* isolates alongside four *C. jacksonii* isolates, indicated with a '*'; introns were not detected in *C. jacksonii*. More information about each isolate can be found in table S2.1. A Thermo Scientific GeneRuler 1 kb DNA ladder was used as the molecular marker for all electrophoresis runs. Reactions containing no template DNA (i.e. blanks) did not produce amplification products.

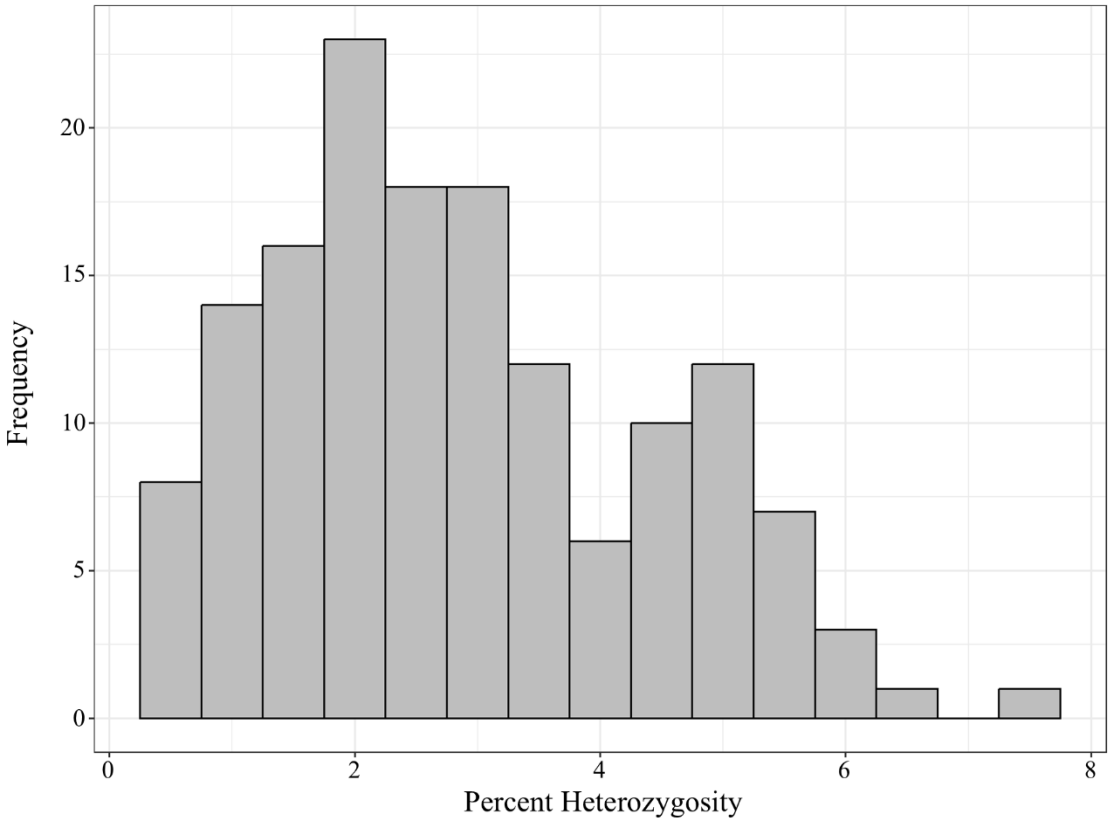


**Figure S2.2:** Histogram depicting the distribution of heterozygosity (%) among *C. monteithiana* isolates sampled from Georgia turfgrasses between 2019 and 2023, ranging from 0.36% to 7.28%.

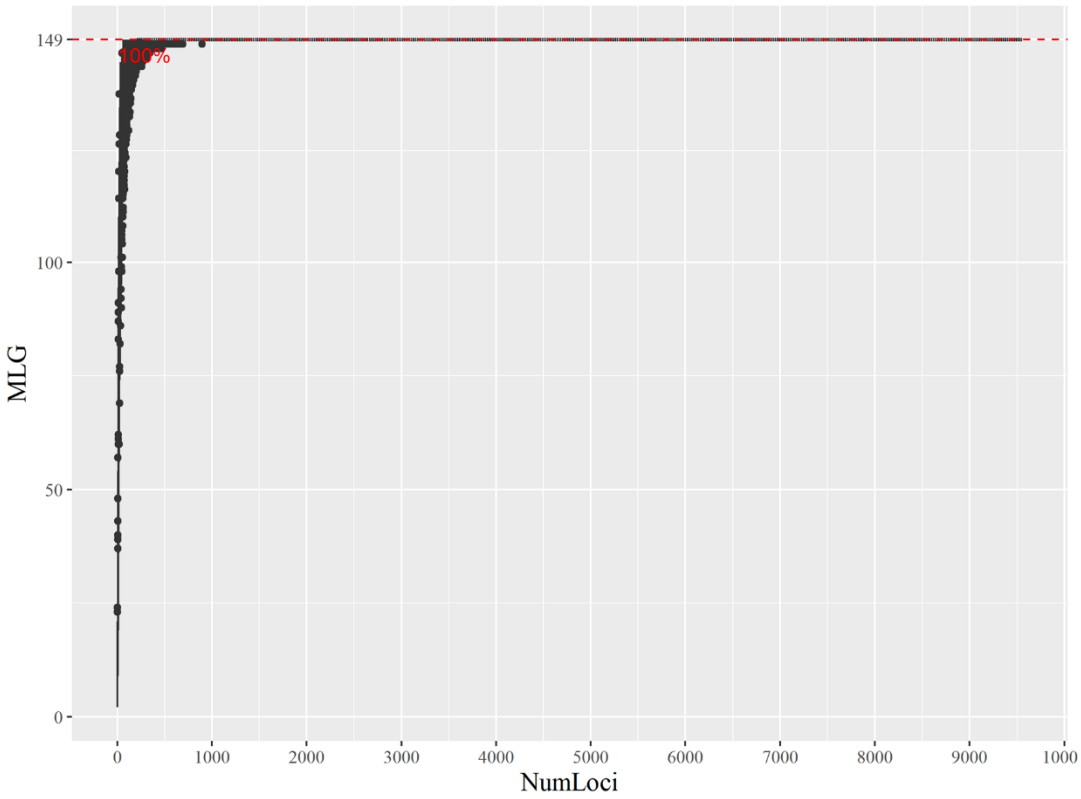


**Figure S2.3:** Genotype accumulation curve for 149 *C. monteithiana* isolates sampled from Georgia turfgrasses from 2019 to 2023, generated under the *poppr* package in R v4.3.2. All isolates represented a unique MLG, and all 149 MLGs could be distinguished by fewer than 1,000 loci.

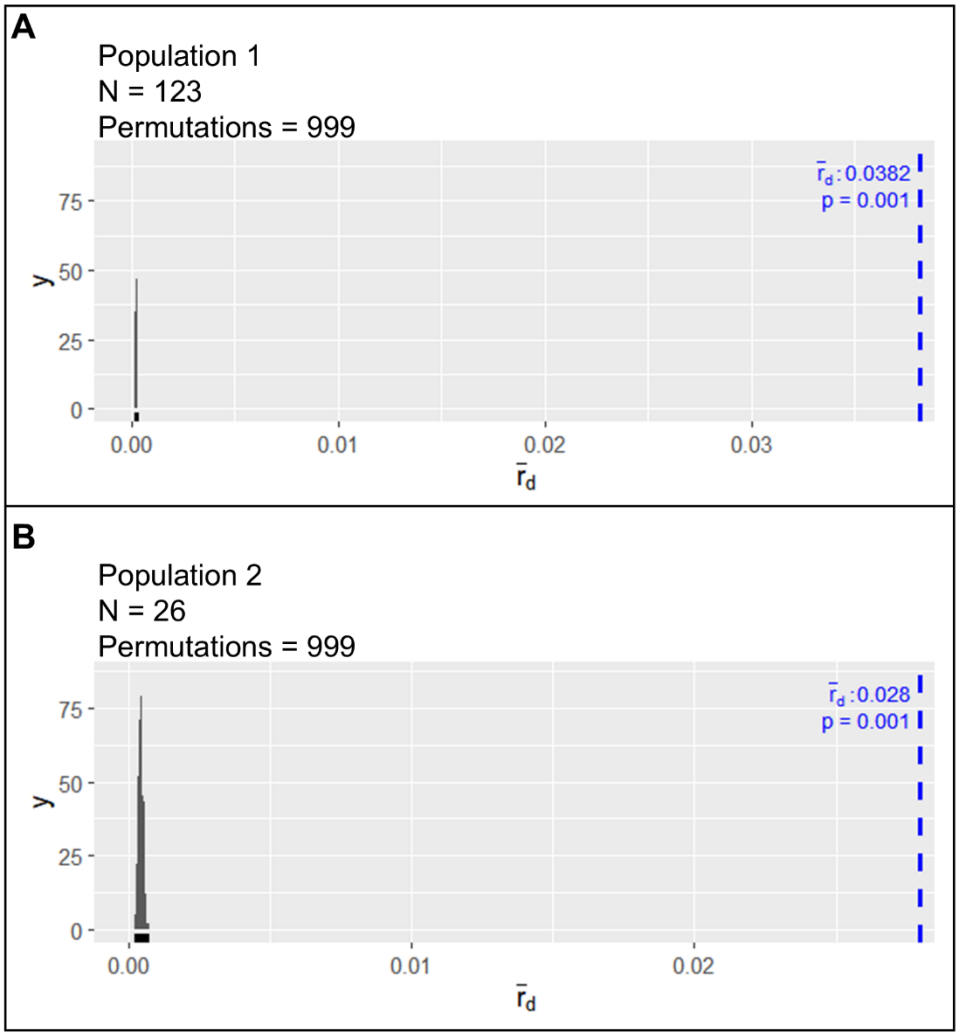


**Figure S2.4:** Distributions of standardized index of association for *C. monteithiana* population 1 (A) and population 2 (B) based on 999 permutations of GBS data, generated using the *poppr* package in R v4.3.2. The observed values are indicated by the blue dashed lines; the expected distribution under random mating is indicated in black bars. Both observed distributions provide evidence to reject the null hypothesis of random mating ($P = 0.001$).

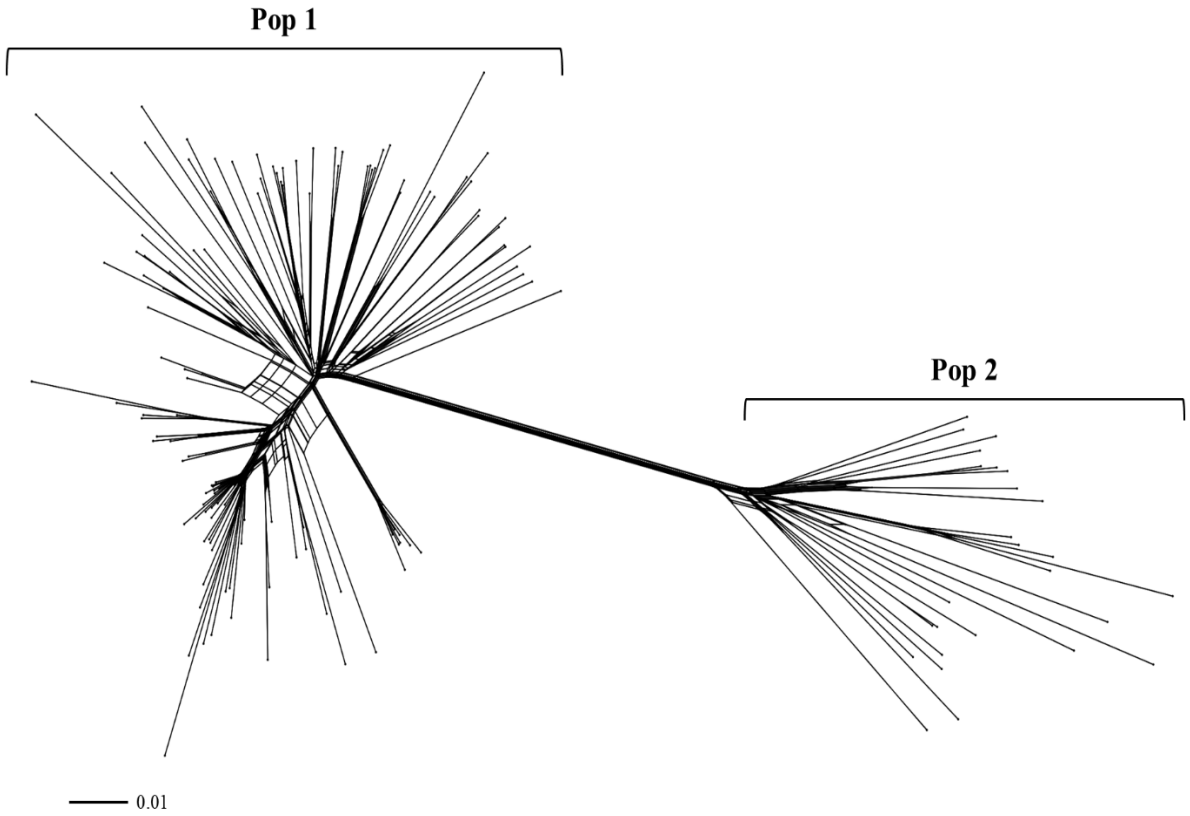


**Figure S2.5:** Neighbor-net phylogenetic network for 149 *C. monteithiana* isolates sampled from Georgia turfgrasses from 2019 to 2023, constructed in SplitsTree v6.3.41 using GBS marker data. Regions of phylogenetic signal conflict are indicated by network reticulations (box-like structures).

**Note**: Supplemental Table S2.2 (GBS dataset used for the genetic diversity and population structure analyses, comprised of 11,477 SNPs and 149 *C. monteithiana* isolates sampled from Georgia turfgrasses from 2019 to 2023.) is not included in this Dissertation Chapter 2.

# CHAPTER 3

# ASSESSING UV-C RADIATION TREATMENTS FOR DOLLAR SPOT SUPPRESSION IN SEASHORE PASPALUM

Willis T. Spratling, Paul L. Raymer, Qianqian Fan, Somerville Rowe, David Jespersen, Clint Waltz, Alfredo D. Martinez-Espinoza and Bochra A. Bahri. Submitted to *Plant Disease*

**Abstract**

Dollar spot, caused by *Clarireedia* spp., is one of the most detrimental diseases of turfgrass worldwide, and control strategies usually involve frequent fungicide applications. These treatments are expensive, require special equipment, and can contribute to fungicide resistance issues, underscoring the need for alternative management strategies. UV-C radiation has proven effective as a disease management tool in various cropping systems but is still largely unexplored in turfgrass. This study aimed to test the effects of UV-C radiation against dollar spot in seashore paspalum and to evaluate its impact on plant health and performance. In assessing UV-C's efficacy directly against *C. monteithiana*, daily radiation treatments ranging from 25-s to 70-s were shown to effectively reduce mycelial growth. Additionally, *in vitro* UV-C treatment administered in darkness was observed to be more effective in reducing pathogen growth than treatment administered in lighted conditions. In a growth chamber setting, daily 60-s UV-C treatment significantly reduced dollar spot severity in seashore paspalum without causing phytotoxic damage to plant tissues. In field trials, a novel UV-C application system was implemented by modifying a robotic mower to autonomously deliver UV-C radiation to seashore paspalum plots. UV-C treatment in the field significantly reduced dollar spot severity. Moreover, UV-C treatment led to several physiological and performance enhancements, including increased chlorophyll content, shoot density, surface firmness, and green speed. Findings from this study indicate that UV-C radiation may be used as an effective physical control to complement existing dollar spot management practices.

**Keywords**: Dollar Spot, Ultraviolet-C, *Clarireedia*, Turfgrass, Disease Management

## Introduction

UV (ultraviolet) light falls between visible light and X-rays on the electromagnetic spectrum and encompasses electromagnetic radiation with wavelengths ranging from 100 to 400 nm (Guerrero-Beltran and Barbosa-Canovas, 2004). The UV region is typically subdivided into three bands: UV-A, UV-B, and UV-C. Of these bands, UV-C light has the shortest wavelengths (100 to 280 nm) and thus the highest energy (Stapleton, 1992). Earth's stratospheric ozone layer absorbs most of the UV-C radiation from the sun and prevents it from reaching the planet's surface (Caldwell et al., 1989). Therefore, sources of UV-C radiation on earth are predominantly artificial, typically generated using light-emitting diode (LED), pulsed-xenon, mercury-vapor, or excimer lamps (Demeersseman et al., 2023).

UV-C radiation causes damage to eukaryotic and prokaryotic microorganisms primarily through altering the structure of their DNA (Reed, 2010). Direct absorption of UV-C radiation by nucleotide bases in DNA can result in the development of molecular lesions, which typically manifest as pyrimidine dimers (Setlow and Carrier, 1966). The most well-characterized pyrimidine dimers induced by UV-C radiation include cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone photoproducts (6-4PP) (Rastogi et al., 2010). Of these, CPDs are more common and occur when covalent bonds form between carbons five and six of two adjacent pyrimidines in a DNA strand (Rochette et al., 2006; Setlow, 1966). If not repaired, CPDs and other molecular lesions impair the ability of polymerases to recognize or navigate bases during replication (Edenberg, 1976; Harm, 1980). This replication interference can lead to cell cycle arrest, which in turn disrupts cellular growth and reproduction (Chastain II et al., 2006; Gentile et al., 2003). Additionally, apoptosis may be triggered if UV-C damage is severe or prolonged (Godar, 1996). Other harmful effects UV-C radiation can produce in living cells

include lipid peroxidation, enzyme inactivation, protein polymerization, and increased cell membrane permeability (Mandal and Chatterjee, 1980; Meffert et al., 1976; Wuytack et al., 2003).

Given its strong genotoxic nature, UV-C radiation has been utilized for disinfection purposes across several industries including healthcare, food safety, water treatment, agriculture, and others (Chatzisymeon et al., 2011; Singh et al., 2021; Urban et al., 2016; Yang et al., 2019). In the agricultural sector, several studies have shown that UV-C treatment can have a direct antagonistic effect against plant pathogens. For instance, in a study assessing the efficacy of UV-C radiation against the strawberry (*Fragaria* × *ananassa* Duch.) gray mold pathogen (*Botrytis cinerea*), Janisiewicz et al. (2016a) found that administering a dose of 12.36 J $m^{-2}$, followed by a period of darkness, led to the near complete kill of *B. cinerea* conidia grown on agar media. In greenhouse and leaf disk assays, the same UV-C treatment also led to near total kill of the strawberry powdery mildew pathogen (*Podosphaera aphanis*) (Janisiewicz et al., 2016b). Weekly applications of this UV-C treatment on strawberry plants showed no adverse effects on photosynthesis, pollen viability, or fruit yield (Janisiewicz et al., 2016a). Furthermore, in evaluating UV-C applications for mold diseases of orange (*Citrus sinensis*), Gündüz and Pazir (2013) reported that *in vitro* treatments (0.84 kJ $m^{-2}$) significantly reduced the growth of *Penicillium italicum* and *P. digitatum* by 82.5% and 56.2%, respectively, compared to controls. After treating inoculated oranges with a higher UV-C dose (7.92 kJ $m^{-2}$), they observed a threefold decrease in infection rates for both pathogens compared to controls (Gündüz and Pazir, 2013). While these studies and others demonstrate UV-C's efficacy against fungal plant pathogens, several more have documented its germicidal activity against a wide array of bacterial

plant pathogens, underscoring its broad-spectrum potential in disease management (Escalona et al., 2010; Fan et al., 2017; Gayán et al., 2013; Syamaladevi et al., 2013).

In addition to directly suppressing plant pathogens, UV-C treatments have also been shown to stimulate plant defenses. Although mechanisms of induced plant defense from UV-C treatments are not fully understood, upregulations of specialized plant metabolites such as phenolics, terpenes, and nitrogen-containing compounds, along with defense hormones like salicylic acid and jasmonic acid, have been observed and are believed to contribute to pathogen resistance (Vanhaelewyn et al., 2020). For example, after treating greenhouse lettuce (*Lactuca sativa* L.) with UV-C (0.85 kJ $m^{-2}$) four times over two-day intervals, Vàsquez et al. (2017) observed notable increases in total phenol content and phenylalanine ammonia lyase activity in leaves. After inoculating these leaves with *B. cinerea* two days post-treatment, the authors reported a significant 30% decrease in disease severity over the course of the trial compared to controls (Vàsquez et al., 2017). Similarly, in greenhouse trials, Aarrouf and Urban (2020) found that administering UV-C light flashes (1 kJ $m^{-2}$) two days before pathogen inoculation significantly reduced the severity of *Phytophthora capsici* in pepper (*Capsicum annuum* L.), *Plasmopara viticola* in grape (*Vitis vinifera* L.), and *B. cinerea* in lettuce and tomato (*Solanum lycopersicum* L.). They also found that repeated UV-C treatments over the course of several days were more effective in reducing disease than a single treatment (Aarrouf and Urban, 2020). Other reports of UV-C-induced plant disease resistance have been documented for peach (*Prunus persica* (L.) Batsch), sweet potato (*Ipomoea batatas* (L.) Lam), and apple (*Malus domestica* Borkh.) against *Monilinia fructicola*, *Fusarium* spp., and *Alternaria* spp. pathogens, respectively (Lu et al., 1991; Stevens et al., 1999; Stevens et al., 1998).

While UV-C radiation has proven effective against plant pathogens and in enhancing plant defenses, most studies have been confined to fruit and vegetable cropping systems. Information on the effectiveness of UV-C in turfgrass health and disease management is particularly limited. However, the unobstructive growth habit and topography of turfgrass may allow for improved UV-C exposure and coverage compared to other crops, making it an ideal subject for exploring this technology. As the most widely used groundcover in the United States, turfgrass spans approximately 50 million acres across the country, and the turfgrass industry itself is valued at over US$100 billion (National Turfgrass Federation, 2017; Shaddox et al., 2022). The economic significance and expansive acreage of the crop reflect the strong demand for high-quality turfgrass. A fungal disease that often impedes meeting this demand is dollar spot. Dollar spot reduces playability and aesthetic value of turfgrass stands by causing foliar blighting (Vargas, 2018; Walsh et al., 1999). At least six species within the *Clarireedia* genus have been attributed to causing the disease, with *C. jacksonii* and *C. monteithiana* being the most prominent in the U.S. (Hu et al., 2019; Salgado-Salazar et al., 2018; Zhang et al., 2022). These two species exhibit host specificity, as *C. jacksonii* primarily infects cool-season turfgrasses and *C. monteithiana* primarily infects warm-season turfgrasses (Salgado-Salazar et al., 2018).

To manage dollar spot, turfgrass practitioners often rely on repeated fungicide applications. On golf courses alone, superintendents may make up to ten or more applications per year to achieve adequate control, which can result in annual costs exceeding $10,000 (Bekken et al., 2022; Koch et al., 2021). This management strategy is not only expensive but may also contribute to fungicide resistance issues (Lucas et al., 2015). Dollar spot pathogens can develop resistance to fungicides rapidly, and resistance or insensitivity has already been confirmed in each of the four major fungicide classes used for control, which include the benzimidazoles,

demethylation inhibitors, succinate dehydrogenase inhibitors, and dicarboximides (FRAC, https://www.frac.info/home) (Bishop et al., 2008; Ghimire et al., 2023; Jo et al., 2008; Popko et al., 2018; Stephens and Kaminski, 2019). Moreover, as global fungicide regulations tighten, chemical control options continue to dwindle, further emphasizing the need for alternative management solutions (Gullino and Kuijpers, 1994). In this study, we 1) evaluated the effects of repeated UV-C radiation applications on turfgrass health and performance, and 2) assessed the impacts of these treatments against dollar spot in laboratory, growth chamber, and field settings.

## Materials and methods

### Plant material

Seashore paspalum (*Paspalum vaginatum* Swartz) cv. 'SeaStar' was used in all growth chamber and field trials. Seashore paspalum is a warm-season turfgrass that requires relatively low fertilizer and pesticide inputs and is renowned for its high salt tolerance (Duncan and Carrow, 2000). The variety SeaStar was released in 2011 by the University of Georgia and is characterized by its dark green color, medium-to-fine leaf texture, and exceptional shoot density (Raymer et al., 2014; Schwartz et al., 2013). For growth chamber trials, turfgrass plugs were extracted from an established SeaStar field located at the University of Georgia, Griffin Campus, Griffin, GA. Native soil and sand were cut away from plugs, and they were planted into 7.6 cm × 7.6 cm nursery pots (HC Companies, OH, USA) filled with potting mix (Metro Mix 852; Sun Gro Horticulture, MA, USA). Plants were then transferred to a greenhouse (24 to 32˚C, 78% relative humidity) where they were left to establish for one month. During establishment, plants were well-watered, regularly trimmed to maintain a canopy height of 2.5 cm, and fertilized on a biweekly basis with 3.7 g L Miracle-Gro Water Soluble All-Purpose Plant Food (NPK 24-8-16)

(The Scotts Company LLC, USA). Additionally, plants were routinely checked and culled to ensure healthy, disease-free material was used in growth chamber trials.

The field of seashore paspalum cv. SeaStar from which turfgrass plugs were collected for growth chamber trials was used in all field trials. The field was established on the University of Georgia, Griffin Campus, Griffin, GA in 2016 following United States Golf Association (USGA) green construction specifications (USGA Green Section Staff, 2004). During trials, no pesticides or fertilizers were applied to the field, and turfgrass was mowed daily at a height of 0.3175 cm. Weeds were removed manually as needed, and irrigation ran to supply the field with 2.5 cm of water per week.

**Fungal material and inoculum preparation**

A dollar spot isolate (DS8) collected from a seashore paspalum field located at the University of Georgia, Griffin Campus, Griffin, GA was utilized in all *in vitro* UV-C trials, as well as in UV-C growth chamber experiments that required artificial inoculation. The isolate was collected in 2019 and was stored in a sterile grain mixture of oat, barley, and wheat at -20˚C and in a Microbank vial (Pro-Lab Diagnostics, CA) at -80˚C. This isolate was molecularly identified as *Clarireedia monteithiana* by Sapkota et al. (2020) based on species-specific SNPs within the internal transcribed spacer (ITS) region (ITS sequence stored under NCBI GenBank accession 'MT497854') (Salgado-Salazar et al., 2018).

To prepare cultures for *in vitro* trials, a seed of DS8 grain was retrieved from storage and plated onto potato dextrose agar (PDA) media, and the isolate was allowed to grow under 12-hour light at room temperature for five days. After five days, 3 mm plugs were taken from the culture and placed upside down in the center of fresh, individual PDA media plates (R80085;

Thermo Scientific Inc., Madison, WI). Plates were then sealed and were ready for use in *in vitro* trials.

Artificial inoculum used in growth chamber experiments was created following protocols from Steketee et al. (2016). Briefly, a seed of DS8 storage grain was plated onto PDA media and grew under 12-hour light for seven days at room temperature. Meanwhile, an Erlenmeyer flask (1000 ml) was filled with a 200 ml by volume equal mixture of oat, barley, and wheat. Sterile water was then added to the grain mixture and was left to soak overnight. The flask containing the grain mixture was then autoclaved once daily over two consecutive days. After autoclaving, five 2-$cm^2$ pieces of DS8-infested PDA media were added to the flask to create the inoculum. The inoculum flask was shaken once daily to promote uniform distribution of the pathogen throughout the grain mixture. After two weeks, the inoculum was suitable for use in growth chamber experiments.

**UV-C light setup used for *in vitro*, growth chamber, and field trials**

Across all trials, administered UV-C doses (i.e. treatments) were calculated by multiplying UV-C radiation intensity ($\mu W\ cm^{-2}$) by exposure time (seconds) (Hassen et al., 2000). A portable UV-C light meter (SDL470; EXTECH Instrument, Nashua, NH) was used throughout trials to monitor and maintain treatment consistency. In growth chamber and *in vitro* trials, radiation intensity was set at 110 $\mu W\ cm^{-2}$, and the treatment setup featured a UV-C lamp array comprised of two rows of UV-C lamps (Klarran LE; Crystal IS, Green Island, NY) (Figure S3.1). These lamps were powered by a regulated direct current power supply (Model 1689; B&K Precision Corp., Yorba Linda, CA), and the lamp array was mounted on aluminum heat sinks that were suspended from a wooden frame.

In field trials, a modified Echo Robotics TM-1000 Turf Mower (ECHO Incorporated, Zurich, IL) was used to apply UV-C treatments (Figure S3.2). The mower, hereafter referred to as the UV-C robot, was converted to emit UV-C radiation by removing cutting heads and mounting a UV-C light (42UVX202H; Ningbo Vealite Illumination Co., CN) to the bottom of its chassis. The light was suspended ~5 cm above the ground (UV-C radiation intensity of ~350 μW $cm^{-2}$) and was wired to a direct current to alternating current inverter (DX-GAX400W; GEARGO, CN). The inverter was wired into a programmable switch timer (TM630A; Yueqing Xinyang Technology Co., CN), which was connected to a 12-volt deep cycle battery. The switch timer allowed the light to turn on at preset times, and the light itself was powered by the battery. To keep the battery charged, it was connected to a solar charge controller (H0911-2; Depvko Co., USA) and a solar panel (RNG-100D-SS-US; Renogy LLC, CA). To keep the UV-C robot charged, it was programmed to automatically dock at a charging station located near the field whenever it was not in active use. The inverter, timer, battery, and solar charge controller were housed in a container that sat atop the robot, and the solar panel was securely positioned on top of the container. During operation, the UV-C robot traveled at a ground speed of 0.72 kph. Overall, this configuration allowed for targeted, autonomous delivery of UV-C radiation to field plots.

**UV-C *in vitro* trials**

Two *in vitro* trials, *in vitro* Trial 1 (IVT1) and *in vitro* Trial 2 (IVT2), were conducted to test the efficacy of daily UV-C radiation treatments against pure cultures of *C. monteithiana*. Both trials involved seven treatments, each having five replicates. The treatments included Control (no UV-C), 10-s (11.0 J $m^{-2}$ $d^{-1}$ UV-C), 25-s (27.5 J $m^{-2}$ $d^{-1}$ UV-C), 35-s (38.5 J $m^{-2}$ $d^{-1}$

UV-C), 50-s (55.0 J $m^{-2}$ $d^{-1}$ UV-C), 60-s (66.0 J $m^{-2}$ $d^{-1}$ UV-C), and 70-s (77.0 J $m^{-2}$ $d^{-1}$ UV-C). Each trial was carried out in a growth chamber (E-41L2; Percival Scientific, IA, USA) set at 23˚ C and 40% relative humidity. The only difference between the two trials was the ambient lighting conditions within the growth chamber. In IVT1, growth chamber lights were kept on (SciWhite™ LED white lighting at 400 µmol $s^{-1}$ $m^{-2}$) for the entire duration of the trial, and they were left off (complete darkness) in IVT2. Additionally, IVT1 and IVT2 were each repeated once (experiments one and two).

*C. monteithiana* (DS8) cultures for *in vitro* trials were prepared as previously described and were randomly arranged within the growth chamber. UV-C treatments started on the same day pathogen cultures were plated, and all treatments were applied at the same time each day in each trial. For every treatment application, plate lids were always removed to ensure pathogens were directly exposed to UV-C radiation. While treatments were administered, the UV-C light array was positioned 8 cm above pathogen cultures.

**UV-C *in planta* growth chamber trials**

Two *in planta* growth chamber trials were carried out to test the effects of daily UV-C radiation on turfgrass tissues and for dollar spot suppression. The initial trial, Growth Chamber Trial 1 (GCT1), was conducted to evaluate whether UV-C treatments had any phytotoxic effects on turfgrass tissues in the absence of disease, as well as to establish a safe UV-C treatment dosage to use in the subsequent growth chamber trial. Seashore paspalum cv. SeaStar pots were acquired and maintained as previously described, and throughout the trial, plants were kept in the same growth chamber used for *in vitro* trials. Growth chamber settings included day and night temperatures of 25˚C and 16˚C, respectively, 100% relative humidity, and a 12-hour

photoperiod (SciWhite™ LED white lighting at 400 µmol $s^{-1}$ $m^{-2}$). The same seven UV-C treatments used in *in vitro* trials were also utilized in this trial (Control, 10-s, 25-s, 35-s, 50-s, 60-s, and 70-s), and treatments were administered one hour after the conclusion of daylight 12-hour photoperiod conditions. Treatments were applied using the same procedures and setup as in *in vitro* trials, with the tops of plant canopies positioned 8 cm below UV-C lamps. The trial was laid out as a completely randomized design (CRD) with five replicated pots per treatment and carried out for a total of 30 days.

The second growth chamber trial, Growth Chamber Trial 2 (GCT2), was conducted to test the efficacy of UV-C radiation in suppressing dollar spot. Based on results from the *in vitro* trials and GCT1, the 60-s UV-C treatment (66 J $m^{-2}$ $d^{-1}$ UV-C) was selected and used in this trial. A control group (no UV-C) was included, and both 60-s and control treatments consisted of eighteen replicated pots. The trial was laid out as a CRD, and all pots in this trial were artificially inoculated with *C. monteithiana*-infested grain (DS8). Grain inoculum was prepared as previously described, and inoculation took place by introducing five infested grain seeds to the foliar canopy of each pot. UV-C treatment started on the same day (just before) plants were inoculated. The same growth chamber and growth chamber settings used in GCT1 were used in this trial, as well as the same UV-C treatment setup and procedures. The trial lasted a total of 30 days and was repeated once (experiments one and two).

**UV-C field trials**

Field trials were conducted to evaluate the impacts of UV-C radiation treatments on turfgrass quality and performance and in the suppression of dollar spot in established turfgrass swards. The first trial, Field Trial 1 (FT1), was held in the growing season of 2020. Seashore

paspalum cv. SeaStar was utilized, and field management practices during the trial were previously outlined. The trial was laid out as a randomized complete block design, and individual field plots measured 3.05 m by 6.09 m. Treatments included UV-C and control groups, each having three replicates. The UV-C robot was used to deliver treatments and operated in UV-C plots from 9 p.m. to 2 a.m. on a nightly basis (~21.0 J $m^{-2}$ $d^{-1}$ UV-C); it did not enter control plots. FT1 lasted from May 4, 2020, until September 9, 2020 (128 days).

Field Trial 2 (FT2) was held in the same field as in 2020 and ran in the growing season of 2023 from August 3 until October 12 (70 days). It was laid out as a CRD, and individual field plots measured 3.66 m by 5.49 m. Treatments in this trial included control, UV-C, and traffic-only groups, each having three replicates. The traffic treatment was implemented to separate "traffic effects" (i.e. mechanical effects caused by the robot navigating through/within plots) from UV-C radiation effects. The only difference between traffic and UV-C treatments was that the robot operated in traffic plots with the UV-C light turned off and in UV-C plots with the light on. The robot ran daily from 9 p.m. to 2 a.m. in UV-C plots (~21.0 J $m^{-2}$ $d^{-1}$ UV-C), from 9 a.m. to 2 p.m. in traffic plots, and it did not enter control plots.

### *In vitro* trial data collection

Mycelial growth in *in vitro* trials was evaluated by measuring fungal colony diameters in two perpendicular directions daily. Measurements were taken with a digital caliper, and the two measurements for each day were averaged to attain a single colony diameter value for each plate (Gandomi et al., 2009). Measurements started the day after plates were inoculated (D01) and lasted until all colony diameters reached the maximum diameter of petri dishes (85 mm).

**Plant disease severity data collection**

In GCT2 and in both field trials (FT1 and FT2), disease severity was assessed by visually determining the percent pot or plot area blighted by dollar spot (0 to 100% linear scale, where 0 = turfgrass area entirely asymptomatic and 100 = turfgrass area entirely symptomatic) (Putman and Kaminski, 2011). Disease scoring commenced on the first day of each trial (D00), and ratings were taken every three days in GCT2 and on a roughly weekly basis in field trials. Area under the disease progress curve (AUDPC) was calculated from blight percentages according to the following formula:

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where $t_i$ is time (day) at the $i^{th}$ observation, $y_i$ is an assessment of disease severity (% turfgrass area blighted) at the $i^{th}$ observation, and $n$ is the total number of observations (Madden et al., 2007).

**Turfgrass quality and performance data collection**

In growth chamber trials, turfgrass quality was assessed through measurements of visual turfgrass quality, green coverage, and Dark Green Color Index (DGCI). These measurements were first recorded on D00 in each trial and were taken every three days thereafter. Visual turfgrass quality scoring was based on factors such as uniformity, density, and color. The National Turfgrass Evaluation Program 1 to 9 quality rating scale, where 1 = completely dead turfgrass, 6 = minimally acceptable turfgrass, and 9 = dark green dense turfgrass, was used to assess visual turfgrass quality (Morris and Shearman, 2008). DGCI is a measurement of color, and green coverage represents the percentage of a given area (i.e. pots or plots) that is covered by

green turfgrass. These ratings were obtained by taking pictures of pots and analyzing images using Field Analyzer software (https://www.turfanalyzer.com/field-analyzer) (Field Analyzer, AR, USA). During image analysis, default settings were used for DGCI, and settings for green coverage included low brightness of 0, high brightness of 90, low saturation of 15 to 20, high saturation of 100, low hue of 35 to 45, and high hue of 360. All images were taken using a digital camera (Powershot G9x Mark II; Canon Inc., NY, USA) that was mounted to a lightbox to maintain consistent lighting conditions.

In field trials, seven different parameters were measured to assess turfgrass quality and performance. These included: green coverage, Normalized Difference Vegetation Index (NDVI), total chlorophyll content, vegetative shoot density, clipping weight, ball roll distance, and surface firmness. Green coverage was recorded approximately weekly by taking pictures of the turfgrass surface in three random locations within each plot using the same camera and lightbox used in growth chamber trials. Images were analyzed using Field Analyzer with settings at 54 for low hue, 360 for high hue, 0 for low brightness, 100 for high brightness, 10 for low saturation, and 100 for high saturation. Green coverage values from the three pictures were averaged to attain a single value per plot. NDVI was measured approximately weekly by making three passes through each plot with a handheld NDVI meter (GreenSeeker Handheld Crop Sensor; Trimble Agriculture, CO, USA). NDVI values from each pass were averaged together to attain a single value per plot (Bell et al., 2009).

Total chlorophyll content was measured two times in 2020 and four times in 2023 by pooling approximately 0.1 g leaf tissues from five sub-sampled locations within each plot and incubating tissues in 5 mL dimethyl sulfoxide for seven days to extract chlorophyll. The absorbance of solutions at 665 and 649 nm were measured using a spectrophotometer (Evolution

300 UV-visible spectrophotometer; Thermo Scientific, Madison, WI) to calculate chlorophyll contents on a dry weight basis (Wellburn, 1994). Shoot density was recorded by manually counting the number of individual vegetative shoots present at three randomly sampled areas within each plot (Jordan et al., 2003). Samples consisted of turfgrass plugs extracted with a soil probe, and shoot density was calculated by dividing the number of shoots by the cross-sectional area of the probe (5.1 $cm^2$ in 2020 and 14.5 $cm^2$ in 2023). Samples for shoot density were taken three times in 2020 and four times in 2023. To gauge turfgrass growth, mower clippings were collected and weighed for three weeks in 2020. However, due to equipment failures, clippings were not collected in 2023. During designated collection weeks, clippings were harvested from a single pass of a greens mower (Eclipse 2 Hybrid; Textron Specialized Vehicles, GA, USA) (55.9 cm wide) over the entire length of each plot for five consecutive days (Monday through Friday). Collected clippings were placed in paper bags and dried in a forced air oven (LO-850-P; Blue M, PA, USA) at 50˚C for 48 hours. After drying and cooling, clippings were then weighed. Weights recorded across the five collection days were averaged to establish a mean weekly clipping weight for each plot (Ervin and Koski, 2001).

To assess green speed, a USGA Stimpmeter was used to measure ball roll distance according to the manufacturer's instructions (Radko, 1980). Two Stimpmeter readings were taken from opposite parallel directions in each plot for a total of four readings per plot, and these readings were averaged together to provide a single ball roll distance estimate for each plot. To maintain consistency in evaluating ball roll distance, small reference points were marked in every plot to ensure measurements were taken from the same locations every time. Ball roll distance was measured three times in 2020 and four times in 2023. Lastly, surface firmness ratings were collected using a USGA TruFirm Turf Firmness Meter (USGA, NJ, USA)

(Menchyk et al., 2014; USGA Green Section Staff, 2009). The meter features a hemispherical hammer that is designed to replicate the impact force and inertia of a golf ball striking a turfgrass surface. Penetration depth of the hammer corresponds to surface firmness, with smaller penetration values representing a firmer surface. Firmness readings were taken eight times in 2020 and four times in 2023. Every time firmness data was collected, TruFirm readings were taken from six different locations within each plot, and these readings were averaged together to attain a single probe penetration value for each plot.

**Data analysis**

All trial data were analyzed using RStudio statistical software (R 4.3.2; Boston, MA, USA). Using the *lme4* package, linear mixed-effects models were fit with both 'treatment' and 'day' as fixed effects and 'plate/plot/pot identifier' or 'replication' as random effects. Data were subjected to repeated measures ANOVAs and means were separated using Tukey's HSD test at the 0.05 alpha level. Data from replicate experiments in growth chamber and *in vitro* trials were analyzed separately due to significant effects of 'experiment' or 'treatment × experiment' observed during preliminary analyses (Tables S3.1 and S3.2). 2020 (FT1) and 2023 (FT2) field data were also analyzed separately due to the different treatments and experimental designs (Table S3.3). All figures were created in RStudio using various functions in *ggplot2*, *ggforce*, and *ggpubr* packages.

## Results

### UV-C *in vitro* trials (IVT1 and IVT2)

Significant effects of 'treatment' and 'treatment × day' on mycelial growth were observed in each *in vitro* experiment conducted (Table S3.1). In experiments one and two of IVT1, daily UV-C radiation treatments of 50-s, 60-s, and 70-s resulted in significant reductions in colony diameter compared to the control (Figure 3.1). In experiment one, these treatments led to 15.3% (50-s), 13.3% (60-s), and 18.4% (70-s) reductions in colony diameter compared to the control, and the same treatments in experiment two provided 13.6% (50-s), 18.4% (60-s), and 26.1% (70-s) reductions ($P < 0.0001$). The effects of 10-s, 25-s, and 35-s treatments on colony growth did not result in significant differences relative to the control treatment over the course of either experiment ($P > 0.14$). Furthermore, analysis conducted on daily measurement data revealed significant differences ($P < 0.05$) among treatments occurring on five out of ten days in experiment one and six out of ten days in experiment two (Figure S3.3).

In both experiments of IVT2, all daily UV-C radiation treatments of at least 25 seconds led to a significant reduction in colony diameter compared to the control (Figure 3.1), with significant differences ($P < 0.05$) among treatments occurring on fifteen out of twenty days in experiment one and nineteen out of twenty days in experiment two (Figure S3.3). In experiment one, colony diameters measured 13.3%, 17.3%, 21.6%, 21.6%, and 28.7% less than the control for treatments 25-s, 35-s, 50-s, 60-s, and 70-s, respectively ($P < 0.0001$). In experiment two, the same treatments produced 14.1%, 15.6%, 21.8%, 28.0%, and 27.2% reductions in colony diameter compared to the control, respectively ($P < 0.0001$). Differences in colony diameter between 10-s and control treatments were not significant across either experiment ($P > 0.31$).

Overall, daily UV-C radiation treatments of 10-s, 25-s, 35-s, 50-s, 60-s, and 70-s administered in dark conditions (IVT2) led to significantly greater reductions in colony diameter ($P < 0.05$) by 36.2%, 95.1%, 85.9%, 69.6%, 78.3%, and 78.5%, respectively, compared to the same treatments administered in lighted conditions (IVT1).

**UV-C *in planta* growth chamber trials (GCT1 and GCT2)**

In GCT1, 10-s, 25-s, 35-s, 50-s, and 60-s daily UV-C treatments had no adverse effect on visual turfgrass quality and did not significantly differ from the control treatment ($P > 0.98$). Visual turfgrass quality scores for treatments 10-s to 50-s remained unchanged from beginning to end of the trial, while treatment 60-s scores varied slightly. Plants treated for 70-s were the only ones that showed a decline in visual turfgrass quality that significantly differed ($P = 0.0006$) from the control (Table 3.1). A similar trend was observed in green coverage scoring, where the 70-s treatment caused a statistically significant decline ($P = 0.005$) relative to the control treatment (Table 3.1). Furthermore, no UV-C treatment provided significant DGCI differences when compared to the control ($P > 0.07$) (Table 3.1). Overall, UV-C treatments administered in this trial did not cause extensive damage to turfgrass tissues, as evidenced by all treatments maintaining mean scores of at least 8.6, 93.9%, and 0.390 for visual turfgrass quality, green coverage, and DGCI, respectively.

Based on results from *in vitro* trials and GCT1, the 60-s daily UV-C treatment was selected and implemented in GCT2. Significant effects of ‘treatment’ and ‘treatment × day’ were observed for most parameters assessed in each experiment of GCT2 (Table S3.2). In experiment one, UV-C treatment significantly improved turfgrass quality and green coverage by 11.7% ($P = 0.0002$) and 11.3% ($P = 0.0004$), while also significantly reducing dollar spot severity and

AUDPC by 35.4% ($P = 0.0002$) and 34.2% ($P = 0.0003$), respectively, compared to the control (Figure 3.2). UV-C treatment in experiment two led to significant increases of 16.3% ($P = 0.0001$) in turfgrass quality and 11.0% ($P = 0.002$) in green coverage, as well as significant reductions of 42.5% ($P = 0.0002$) in dollar spot severity and 42.3% ($P = 0.0003$) in AUDPC, compared to the control (Figure 3.2). Regarding DGCI, UV-C treatment had no effect relative to the control ($P = 0.16$) in experiment one but caused a significant 2.8% increase ($P = 0.03$) in experiment two (Figure 3.2).

Analysis by scoring day for each experiment of GCT2 revealed that UV-C-treated pots had significantly higher ($P < 0.04$) visual quality ratings than control pots on all days except D00 (Figure S3.4). Similarly, UV-C green coverage scores were significantly ($P < 0.005$) higher than control scores from D09 onward in both experiments (Figure S3.4). In terms of dollar spot severity, UV-C-treated plants exhibited significantly lower ($P < 0.009$) infection than control plants from D06 onward in experiment one and from D03 onward in experiment two (Figure S3.4). Lastly, significant differences ($P < 0.02$) in DGCI between control and UV-C treatment occurred on three out of eleven scoring days in each experiment, with UV-C-treated plants typically maintaining higher scores than non-treated plants across all timepoints (Figure S3.4).

**UV-C field trial 1 (FT1)**

In FT1, a significant effect of 'treatment' type was found for all parameters assessed, and the interaction of 'treatment × day' was significant for all except three parameters (Table S3.3). UV-C treatment significantly reduced overall dollar spot severity and AUDPC by 60.3% ($P = 0.001$) and 73.7% ($P = 0.002$), respectively, compared to the control treatment (Figure 3.3). Throughout the trial, UV-C-treated plots exhibited equal or less dollar spot severity than control

plots on all but one scoring day (D07) (Figure 3.3). Additionally, UV-C treatment led to significantly higher NDVI ($P = 0.006$, 4.5% increase) than the control treatment, as well as significantly higher green coverage ($P = 0.002$, 1.8% increase) (Figure 3.4). In the early stages of the trial, NDVI differences between UV-C and control plots were minor, with no statistically significant differences ($P > 0.12$) occurring until D42. However, from that day forward, UV-C NDVI ratings were significantly higher ($P < 0.02$) than control ratings on every scoring day through the end of the trial (Figure S3.5). A similar trend was observed in green coverage, with differences between UV-C and control treatments becoming more prominent around D49. Following D49, UV-C green coverage ratings were significantly higher ($P < 0.01$) than control ratings on six out of ten scoring days (Figure S3.5). Furthermore, UV-C treatment provided a significant 29.1% ($P = 0.003$) increase in total chlorophyll content over the control (Figure 3.4), with UV-C plots significantly outscoring ($P < 0.004$) control plots on all individual rating dates (Figure S3.5).

Effects of UV-C treatment on shoot density and clipping weight were also statistically significant relative to the control treatment, with UV-C producing a 24.6% ($P = 0.03$) increase in shoot density and a 51.9% ($P = 0.02$) decrease in clipping weight (Figure 3.4). For both these parameters, significant differences ($P < 0.02$) between treatments occurred on two out of three rating dates (Figure S3.5). Moreover, UV-C treatment significantly increased surface firmness and ball roll distance by 19.1% ($P = 0.003$) and 22.6% ($P = 0.007$), respectively, compared to the control (Figure 3.4). For all eight firmness rating dates, UV-C plots had significantly lower ($P < 0.0001$) probe penetration values (i.e. a firmer surface) than control plots (Figure S3.5). Similarly, for all ball roll distance rating dates, measurements were significantly higher ($P < 0.03$) in UV-C-treated plots than in control plots (Figure S3.5).

**UV-C field trial 2 (FT2)**

In FT2, which included UV-C, traffic, and control groups, a significant effect of 'treatment' was observed for all but one parameter assessed, while a significant 'treatment × day' interaction was found for all but three parameters (Table S3.3). Natural dollar spot infection in FT2 was first observed around D42, and every scoring day from D49 to D70 showed significant differences ($P < 0.007$) in dollar spot severity among treatments (Figure 3.5). Overall, UV-C treatment significantly decreased disease severity by 62.0% ($P = 0.002$) and AUDPC by 63.7% ($P = 0.002$) compared to the control and led to significant reductions of 50.3% ($P = 0.02$) in disease severity and 52.5% ($P = 0.02$) in AUDPC relative to the traffic treatment (Figure 3.5). However, in terms of overall NDVI, all three treatments were found to be statistically similar ($P > 0.77$) (Figure 3.6). The only significant NDVI finding over the course of the trial occurred on D14, when UV-C plots outscored traffic ($P = 0.03$) but not control ($P = 0.12$) plots (Figure S3.6). Furthermore, while overall mean green coverage scores among the three treatments were closely grouped (UV-C: 90.0%, traffic: 90.5%, control: 93.0%), statistically significant reductions were observed in both UV-C ($P = 0.0004$) and traffic ($P = 0.001$) plots relative to control plots (Figure 3.6). Significant differences ($P < 0.03$) in green coverage among treatments were observed on seven out of ten scoring days over the trial period (Figure S3.6). Moreover, analysis of chlorophyll content data revealed that UV-C treatment caused a significant 14.3% increase over the control ($P = 0.03$) but had no significant effect compared to traffic treatment ($P = 0.49$); traffic and control treatments were also found to be statistically similar ($P = 0.11$) (Figure 3.6). Over the four collection dates for chlorophyll content, significant differences ($P < 0.05$) among treatments were detected on two occasions, with UV-C plots outpacing control and traffic plots on most days (Figure S3.6).

For shoot density, UV-C treatment led to significant increases over control and traffic treatments by 45.6% ($P = 0.0001$) and 41.5% ($P = 0.0001$), respectively (Figure 3.6), and densities in UV-C plots were significantly higher ($P < 0.002$) than both traffic and control plots on each of the four individual scoring days (Figure S3.6). Lastly, similarly to FT1, UV-C plots were found to be significantly faster (i.e. increased ball roll distance) and firmer than control plots. Ball roll distance measurements were significantly higher ($P < 0.03$) in UV-C plots than in control plots on all four scoring days (Figure S3.6), ultimately resulting in a 23.3% ($P = 0.0001$) increase in green speed (Figure 3.6). Across the four scoring days for surface firmness, UV-C plots had significantly lower ($P < 0.007$) probe penetration values (i.e. a firmer surface) than control plots on three occasions (Figure S3.6), leading to an overall firmness increase of 9.4% ($P = 0.01$) (Figure 3.6). Compared to the traffic treatment, UV-C treatment had a negligible effect on surface firmness ($P = 0.69$) but significantly increased ball roll distance by 7.4% ($P = 0.02$) (Figure 3.6).

**Discussion**

The objective of this study was to evaluate the effectiveness of UV-C radiation as a novel treatment for dollar spot in seashore paspalum and its impact on plant health and performance. Across all *in vitro*, growth chamber, and field trials conducted, daily applications of UV-C radiation reduced *C. monteithiana* growth or dollar spot severity. In *in vitro* trials, daily applications of UV-C radiation significantly suppressed pathogen growth on PDA media by up to 28%. In growth chamber and field trials, daily UV-C treatment significantly reduced dollar spot severity by up to 42% and 63%, respectively. These results uphold findings from several previously cited studies that UV-C treatments effectively reduce fungal pathogen growth *in vitro*

(Gündüz and Pazir, 2013; Janisiewicz et al., 2016a) and fungal disease severity *in planta* (Aarrouf and Urban, 2020; Janisiewicz et al., 2016b).

It is important to note that in all our trials involving seashore paspalum plants, UV-C treatments were applied preemptively, either just before plants were inoculated in growth chamber experiments or before dollar spot symptoms appeared in field trials. Applying UV-C before the onset of infection may be one of the most effective strategies for disease control, given that it is a non-penetrative form of electromagnetic radiation (Koutchma, 2019; Sommers and Cooke, 2009). This characteristic of UV-C makes it less effective in targeting or reaching plant pathogens that have infiltrated deeper plant tissue layers (Charles et al., 2010; Manzocco et al., 2011; Otake et al., 2021). This premise was substantiated by Gündüz and Pazir (2013), who tested UV-C against *Penicillium* pathogens that had been introduced to oranges via different inoculation methods. They found that UV-C treatments were less effective when pathogen spores were inoculated deeper into tissues through wounding or piercing methods as opposed to when they were spread over tissue surfaces (Gündüz and Pazir, 2013). This emphasizes the importance of timing UV-C applications to target pathogens early, ideally before they establish within plant tissues. Moreover, yet another advantage of early UV-C treatments is the potential to stimulate plant defenses, as previously alluded to (Aarrouf and Urban, 2020; Urban et al., 2018; Vanhaelewyn et al., 2020). Subjecting plants to low-grade stressors like UV-C prior to pathogen exposure can enhance their ability to mount a more robust defense response against subsequent challenges (Martins et al., 2022; Mauch-Mani et al., 2017). This process, sometimes referred to as defense priming, usually does not provide complete disease protection but offers broad-spectrum effectiveness with minimal fitness costs (Tiwari et al., 2022). Answering whether UV-

C induced defense priming is triggered in seashore paspalum was outside the scope of this study but warrants further investigation.

Furthermore, we observed that UV-C treatment efficacy may be influenced by ambient lighting conditions. Specifically, *in vitro* UV-C treatments of 50-s or longer significantly reduced pathogen growth relative to the control in illuminated incubation conditions, while just 25-s treatments achieved similar results in dark conditions. This increased potency of UV-C against fungal pathogens in dark environments is corroborated by other studies and may be attributed to mechanistic limitations in fungal DNA repair, particularly in photorepair (Janisiewicz et al., 2016a; Zhu et al., 2018). Photorepair, or photoreactivation, is one of the most common DNA repair mechanisms in fungi and involves photolyase enzymes that directly remedy UV-induced molecular lesions (Tong and Feng, 2022). However, these enzymes require visible light to function, making photoreactivation processes inoperative in dark conditions (Berrocal-Tito et al., 2007; Palmer et al., 2018). Other DNA repair mechanisms for UV-induced molecular lesions that do not require visible light, like nucleotide excision repair, exist in fungi but are typically slower and less efficient (Tong and Feng, 2022). Therefore, nighttime UV-C treatments against *Clarireedia* pathogens may facilitate less opportunity for DNA repair and greater accumulation of DNA damage than daytime treatments.

In addition to reducing pathogen growth and disease severity, other beneficial effects were observed from UV-C treatments across both growth chamber and field trials. In growth chamber experiments, daily 60-s UV-C treatment did not cause phytotoxicity to turfgrass tissues in the absence of disease. Multiple studies in various cropping systems align with our results in that UV-C delivered at optimal doses does not damage plants, and some even report that UV-C treatments confer physiological improvements (Darras et al., 2015; Vàsquez et al., 2017). For

example, UV-C radiation (doses ranging from 3-40 kJ $m^{-2}$) delayed ripening and decreased softening in tomato and peach fruits, both of which are beneficial in produce handling and processing (Maharaj et al., 1999; Stevens et al., 1998; Tiecher et al., 2013; Yang et al., 2014). Enhancements in seed germination, antioxidant capacity, and fruit set from UV-C radiation have also been observed in wheat (*Triticum aestivum* L.), strawberry, and tomato crops, respectively (Darras et al., 2020; Li et al., 2019; Semenov et al., 2020). Furthermore, in the only other UV-C study conducted in seashore paspalum, Fan et al. (2024) found that UV-C applications delivered on a daily basis for as little as six seconds (18 J $m^{-2}$ $d^{-1}$) increased tiller density, chlorophyll levels, and reduced clipping yields of SeaStar plants grown in a growth chamber, compared to untreated controls. Moreover, in field trials, we found that UV-C treatment significantly reduced clipping weight and enhanced NDVI and green coverage in one season compared to the control, while also significantly increasing shoot density across two seasons. The combination of reduced clipping weight and increased shoot density in UV-C plots mirrors the effects produced by plant growth regulators that are commonly used on golf course putting greens to reduce mowing demands (Watschke et al., 1992). In terms of chlorophyll content, chlorophyll levels in UV-C plots were significantly higher than those in control plots in both field trials but were similar to those in traffic plots in FT2. This suggests that both the mechanical action of the robot and UV-C radiation may have affected chlorophyll content. Undue traffic in turfgrass systems (i.e. wear) is usually undesirable because it can lead to tissue tearing, breakage, and abrasion, all of which can result in chlorophyll loss (Trenholm et al., 2001). However, the extent of traffic-induced wear in turfgrass stands depends on many different management, environmental, and plant-related factors (Carrow, 1995). Conversely, some studies have shown that mechanical stimuli can induce mild stress responses in plants that enhance chlorophyll concentration (Biddington, 1986;

Latimer and Mitchell, 1988), and others have indicated that moderate soil compaction may enhance plant root growth and nutrient uptake, thereby promoting chlorophyll synthesis (Alameda and Villar, 2009; Arvidsson, 1999; Tracy et al., 2011). Likewise, UV-C treatment has been shown to boost or sustain chlorophyll levels in various crops, including seashore paspalum (Chairat et al., 2013; Costa et al., 2006; Fan et al., 2024; Kasim and Kasim, 2012). Ultimately, this unanticipated finding in our study necessitates additional testing to elucidate the effects and interactions of traffic and UV-C radiation on chlorophyll content in seashore paspalum.

In terms of performance, the activity of the UV-C robot in traffic and UV-C field plots created faster (i.e. increased ball roll distance) and firmer surfaces compared to the control. From a golf course turfgrass management perspective, faster and firmer greens are often desirable, and the two traits are frequently correlated, with firmer surfaces usually leading to faster greens (Danneberger, 1989). To produce firmer and faster greens, golf course superintendents regularly employ management practices like lightweight rolling to apply pressure and weight to a turfgrass stand (Hartwiger et al., 2001). The UV-C robot utilized in our trials inevitably exerted pressure and weight on field plots during operation. Therefore, while UV-C radiation probably did not directly impact firmness or green speed, the enhancements we observed for these traits are byproducts of how UV-C treatment was applied (i.e. robot traffic effect).

To our knowledge, this is the first report of effective disease control stemming from daily UV-C radiation applications in a turfgrass field setting, as well as the first study to utilize robotic technology to autonomously deliver UV-C radiation treatments to a turfgrass stand. Although the concept of roboticized delivery of UV-C radiation is not completely novel, it has mostly been explored for disinfection purposes in indoor settings like hospitals and food processing plants (Mehta et al., 2023; Yang et al., 2019). This technology is slowly emerging in the agricultural

industry but has primarily been confined to the horticultural sector. In the horticultural space, a few robotics companies such as Saga Robotics, TRIC Robotics, and Advanced Intelligent Systems have designed autonomous UV-C robots that are effective against mildew diseases of strawberry and grape (Gadoury, 2021; Takeda et al., 2021). In the turfgrass industry, companies like SGL and GreensGroomer have designed equipment that emits UV-C radiation, but all of them require a human operator. We propose that the turfgrass industry may benefit from robotic UV-C technology for disease control, physiological enhancements, and improved performance as depicted in our research. We also bring forward that future research is needed to elucidate additional capabilities, possibilities, and limitations of this technology in turfgrass health and disease management.

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**Table 3.1:** Means of visual turfgrass quality, green coverage, and Dark Green Color Index (DGCI) for seashore paspalum 'SeaStar' treated with daily UV-C applications of 10-s, 25-s, 35-s, 50-s, 60-s, and 70-s, along with a control, in Growth Chamber Trial 1.

| **Treatment**[x] | **Turfgrass Quality**[y] | **Green Coverage (%)**[z] | **DGCI**[z] |
|---|---|---|---|
| Control | 9.0[a] | 96.3[ab] | 0.421[ab] |
| 10-s | 9.0[a] | 98.0[a] | 0.431[a] |
| 25-s | 9.0[a] | 98.0[a] | 0.435[a] |
| 35-s | 9.0[a] | 97.5[a] | 0.411[ab] |
| 50-s | 9.0[a] | 96.3[ab] | 0.425[a] |
| 60-s | 8.9[a] | 94.7[bc] | 0.408[ab] |
| 70-s | 8.6[b] | 93.9[c] | 0.390[b] |

[x]Treatments: Control (no UV-C), 10-s (11.0 J $m^{-2}$ $d^{-1}$ UV-C), 25-s (27.5 J $m^{-2}$ $d^{-1}$ UV-C), 35-s (38.5 J $m^{-2}$ $d^{-1}$ UV-C), 50-s (55.0 J $m^{-2}$ $d^{-1}$ UV-C), 60-s (66.0 J $m^{-2}$ $d^{-1}$ UV-C), and 70-s (77.0 J $m^{-2}$ $d^{-1}$ UV-C).

[y]Turfgrass quality was visually scored on a scale of 1 to 9 with 1 being dead turfgrass, 6 being minimally acceptable turfgrass, and 9 being dark green dense turfgrass.

[z]Green coverage and Dark Green Color Index (DGCI) were scored via digital imaging analysis using Field Analyzer.

[y,z]Means followed by the same letter do not significantly differ ($P = 0.05$, Tukey's HSD).

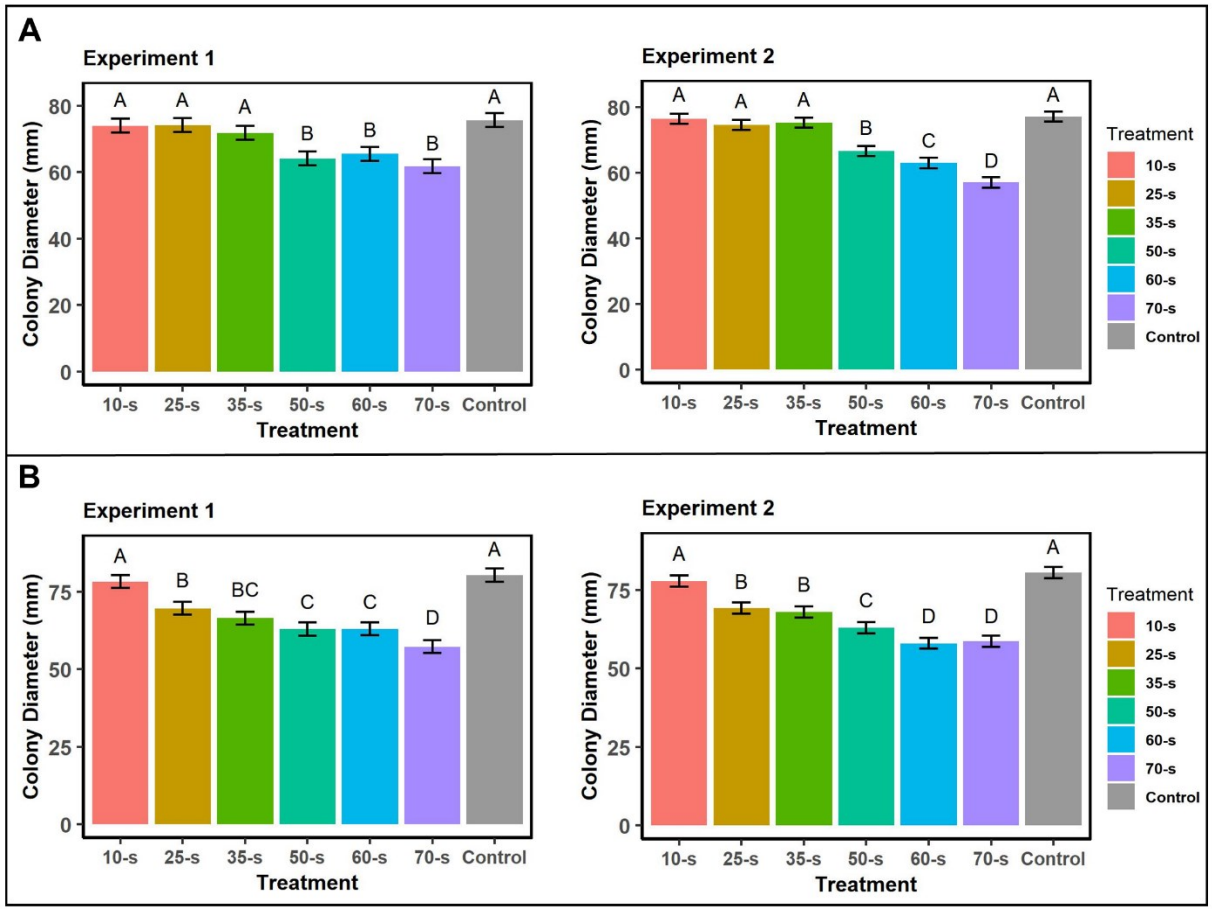


**Figure 3.1:** Means of colony diameter for *Clarireedia monteithiana* (DS8) fungal cultures treated with daily UV-C applications of 10-s, 25-s, 35-s, 50-s, 60-s, and 70-s, along with controls, in *in vitro* trials 1 (A) and 2 (B). *In vitro* trials 1 and 2 were conducted under lighted ambient conditions and complete darkness, respectively. Each trial was replicated once, represented by experiments 1 (left) and 2 (right). Error bars represent standard error. Treatments with the same letter do not significantly differ ($P = 0.05$, Tukey's HSD).

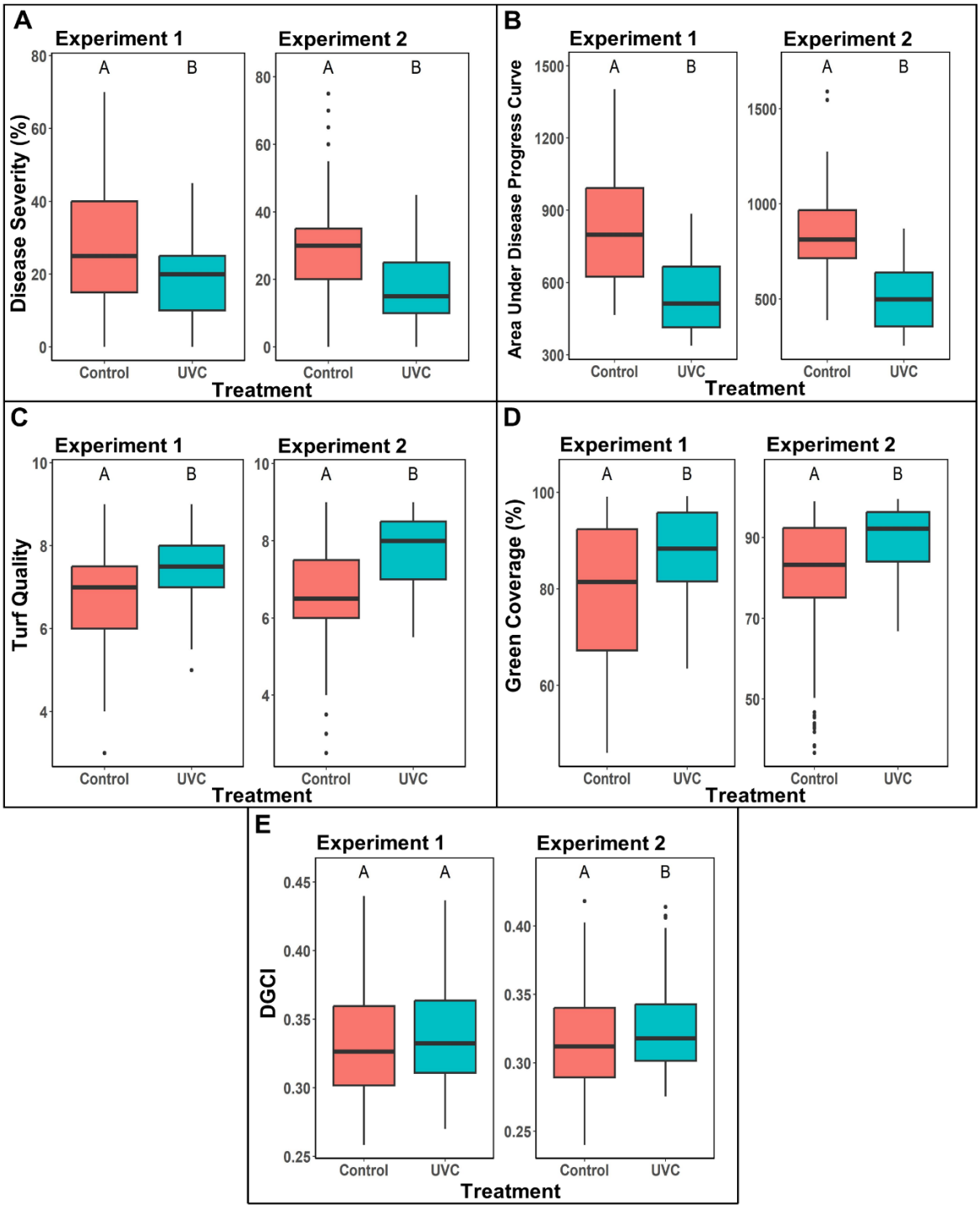


**Figure 3.2:** Boxplots of disease severity (A), Area Under Disease Progress Curve (AUDPC; B), visual turfgrass quality (C), green coverage (D), and Dark Green Color Index (DGCI; E) for dollar spot-inoculated seashore paspalum 'SeaStar' treated with daily 60-s UV-C applications versus a control, in experiments 1 (left) and 2 (right) of Growth Chamber Trial 2. Values for minimum, maximum, median, and first-third quartiles are shown. Treatments with the same letter do not significantly differ ($P = 0.05$, Tukey's HSD).

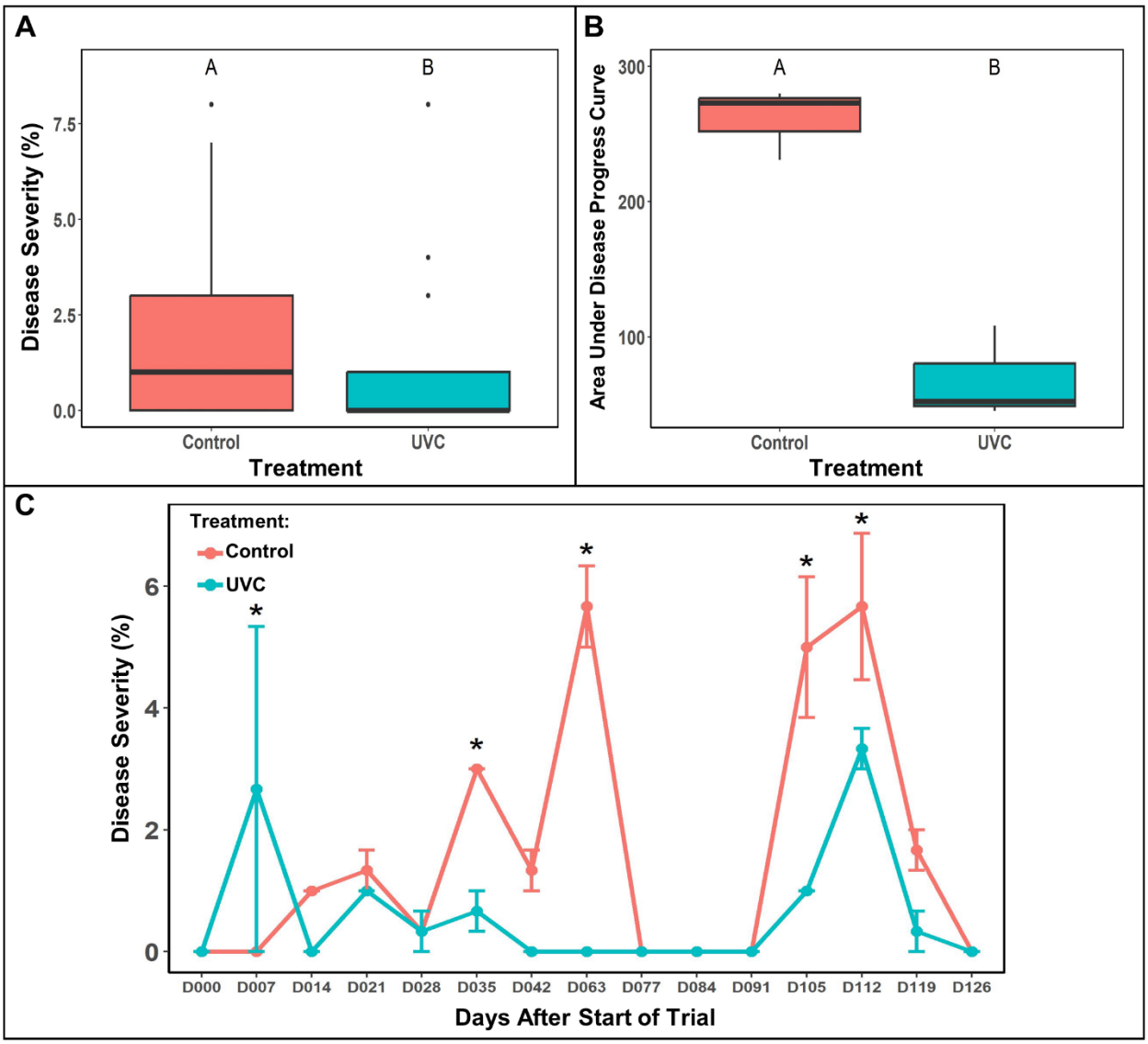


**Figure 3.3:** Boxplots of disease severity (A) and Area Under Disease Progress Curve (AUDPC; B), along with mean disease severity progression (C) for seashore paspalum 'SeaStar' treated with daily UV-C applications versus a control in Field Trial 1. Values for minimum, maximum, median, and first-third quartiles are shown in panels A and B. In panels A and B, treatments with the same letter do not significantly differ ($P = 0.05$, Tukey's HSD). In panel C, error bars represent standard error, and statistical significance between treatments at each timepoint is indicated with '*' ($P = 0.05$, Tukey's HSD).

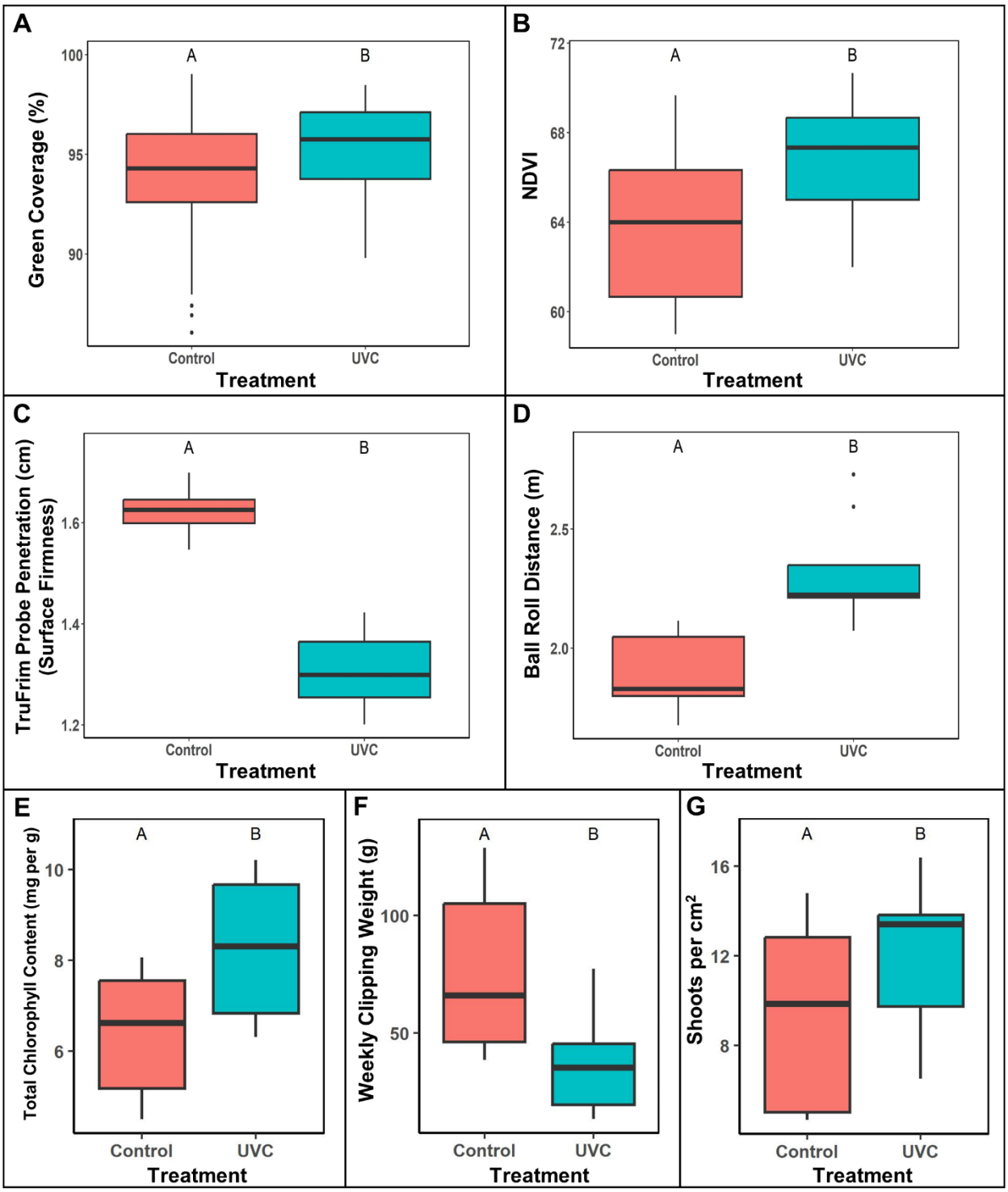


**Figure 3.4:** Boxplots of green coverage (A), Normalized Difference Vegetation Index (NDVI; B), probe penetration (surface firmness; C), ball roll distance (D), chlorophyll content (E), weekly clipping weight (F), and shoot density (G) for seashore paspalum 'SeaStar' treated with daily UV-C applications versus a control in Field Trial 1. Values for minimum, maximum, median, and first-third quartiles are shown. Treatments with the same letter do not significantly differ ($P = 0.05$, Tukey's HSD).

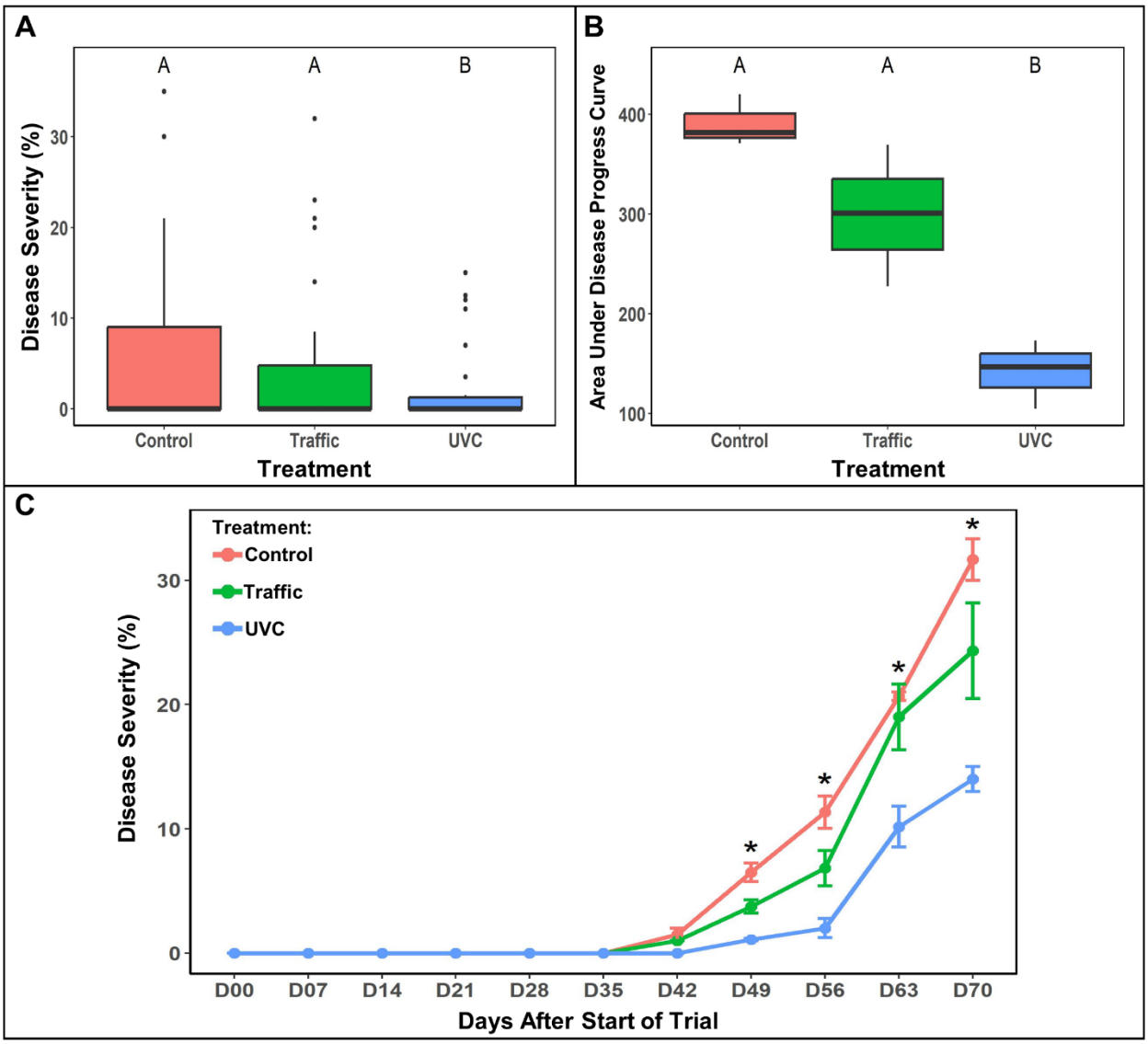


**Figure 3.5:** Boxplots of disease severity (A) and Area Under Disease Progress Curve (AUDPC; B), along with mean disease severity progression (C) for seashore paspalum 'SeaStar' treated with daily UV-C applications versus control and traffic treatments in Field Trial 2. Values for minimum, maximum, median, and first-third quartiles are shown in panels A and B. In panels A and B, treatments with the same letter do not significantly differ ($P = 0.05$, Tukey's HSD). In panel C, error bars represent standard error, and statistical significance between treatments at each timepoint is indicated with '*' ($P = 0.05$, Tukey's HSD).

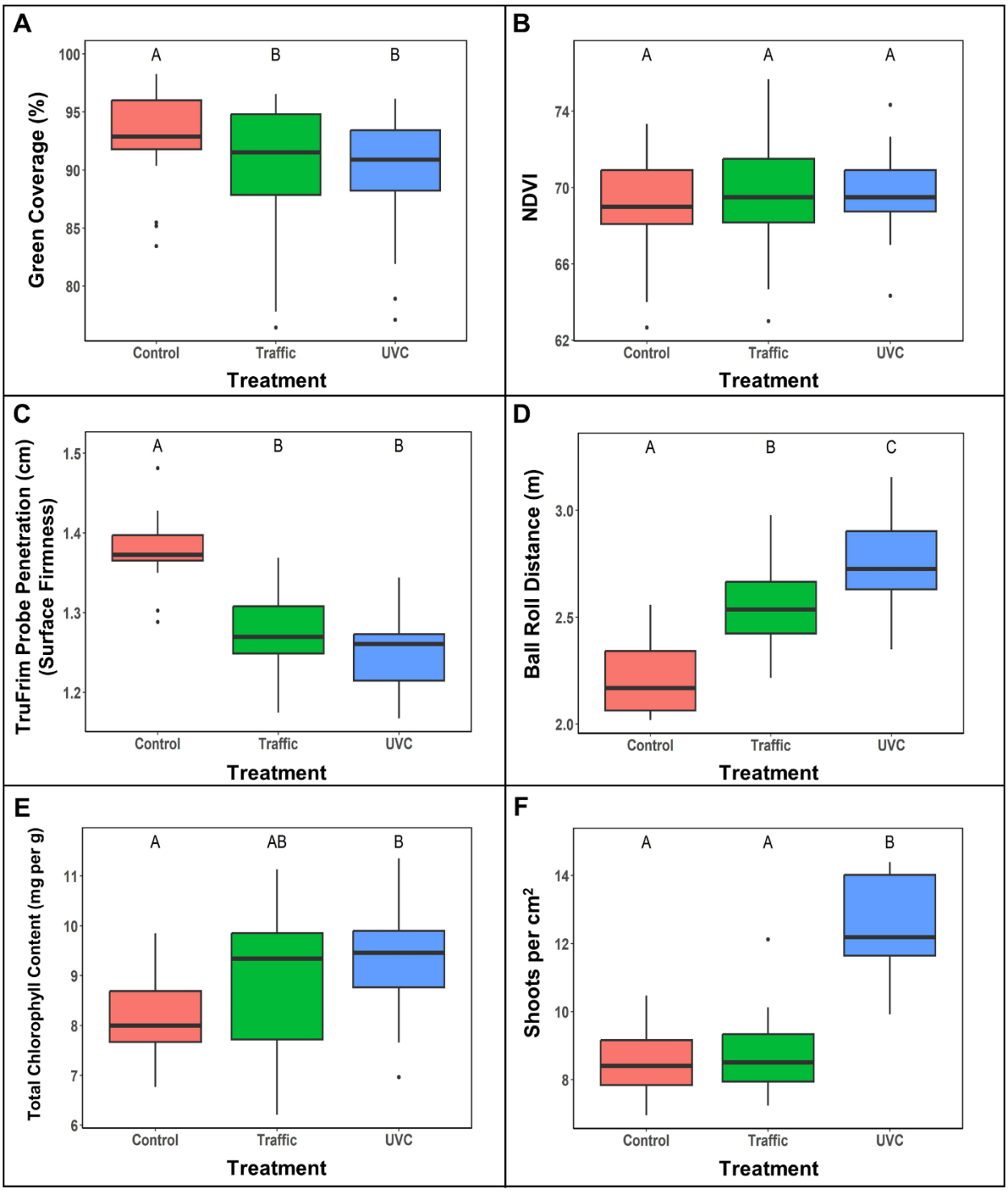


**Figure 3.6:** Boxplots of green coverage (A), Normalized Difference Vegetation Index (NDVI; B), probe penetration (surface firmness; C), ball roll distance (D), chlorophyll content (E), and shoot density (F) for seashore paspalum 'SeaStar' treated with daily UV-C applications versus control and traffic treatments in Field Trial 2. Values for minimum, maximum, median, and first-third quartiles are shown. Treatments with the same letter do not significantly differ ($P = 0.05$, Tukey's HSD).

## Supplemental Materials

**Table S3.1:** *In vitro* trial ANOVA results for colony diameter of *C. monteithiana* (DS8) under UV-C treatments.

| **In Vitro Trial 1 (Experiments Combined)** | | | | | |
|---|---|---|---|---|---|
| | *P value* | | | | |
| *Parameter* | *Treatment* | *Day* | *Treatment × Day* | *Experiment* | *Treatment × Experiment* |
| Colony Diameter | <0.0001 | <0.0001 | <0.0001 | 0.15 | <0.0001 |
| **In Vitro Trial 1 Experiment 1** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | | *Day* | | *Treatment × Day* |
| Colony Diameter | <0.0001 | | <0.0001 | | <0.0001 |
| **In Vitro Trial 1 Experiment 2** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | | *Day* | | *Treatment × Day* |
| Colony Diameter | <0.0001 | | <0.0001 | | <0.0001 |
| **In Vitro Trial 2 (Experiments Combined)** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | *Day* | *Treatment × Day* | *Experiment* | *Treatment × Experiment* |
| Colony Diameter | <0.0001 | <0.0001 | <0.0001 | 0.005 | <0.0001 |
| **In Vitro Trial 2 Experiment 1** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | | *Day* | | *Treatment × Day* |
| Colony Diameter | <0.0001 | | <0.0001 | | <0.0001 |
| **In Vitro Trial 2 Experiment 2** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | | *Day* | | *Treatment × Day* |
| Colony Diameter | <0.0001 | | <0.0001 | | <0.0001 |

**Table S3.2:** Growth chamber trial ANOVA results for measured traits of seashore paspalum under UV-C treatments.

| **Growth Chamber Trial 1** | | | | | |
|---|---|---|---|---|---|
| | *P value* | | | | |
| *Parameter* | *Treatment* | | *Day* | | *Treatment × Day* |
| Visual TQ[a] | 0.0002 | | 0.002 | | <0.0001 |
| Green Coverage | <0.0001 | | 0.0002 | | 0.22 |
| DGCI[a] | 0.003 | | <0.0001 | | 0.96 |
| **Growth Chamber Trial 2 (Experiments Combined)** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | *Day* | *Treatment × Day* | *Experiment* | *Treatment × Experiment* |
| Visual TQ | <0.0001 | <0.0001 | <0.0001 | 0.21 | 0.005 |
| Green Coverage | <0.0001 | <0.0001 | <0.0001 | 0.004 | 0.97 |
| DGCI | 0.01 | <0.0001 | 0.14 | <0.0001 | 0.23 |
| Disease Severity | <0.0001 | <0.0001 | <0.0001 | 0.53 | 0.02 |
| AUDPC[a] | <0.0001 | \ | \ | 0.81 | 0.04 |
| **Growth Chamber Trial 2 Experiment 1** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | | *Day* | | *Treatment × Day* |
| Visual TQ | 0.0002 | | <0.0001 | | <0.0001 |
| Green Coverage | 0.0004 | | <0.0001 | | <0.0001 |
| DGCI | 0.16 | | <0.0001 | | 0.0002 |
| Disease Severity | 0.0002 | | <0.0001 | | <0.0001 |
| AUDPC | 0.0003 | | \ | | \ |
| **Growth Chamber Trial 2 Experiment 2** | | | | | |
| | *P value* | | | | |
| *Parameter* | *Treatment* | | *Day* | | *Treatment × Day* |
| Visual TQ | 0.0001 | | <0.0001 | | <0.0001 |
| Green Coverage | 0.002 | | <0.0001 | | <0.0001 |
| DGCI | 0.03 | | <0.0001 | | 0.44 |
| Disease Severity | 0.0002 | | <0.0001 | | <0.0001 |
| AUDPC | 0.0003 | | \ | | \ |

[a]AUDPC, Area Under the Disease Progress Curve; DGCI, Dark Green Color Index; TQ, Turfgrass Quality

**Table S3.3:** Field trial ANOVA results for measured traits of seashore paspalum under UV-C treatment.

| **Field Trial 1** | | | |
|---|---|---|---|
| | *P value* | | |
| *Parameter* | *Treatment* | *Day* | *Treatment × Day*[b] |
| Green Coverage | 0.002 | <0.0001 | <0.0001 |
| NDVI[a] | 0.006 | <0.0001 | <0.0001 |
| Disease Severity | 0.01 | <0.0001 | <0.0001 |
| Chlorophyll Content | 0.003 | 0.0008 | 0.59 |
| Shoot Density | 0.03 | <0.0001 | 0.09 |
| Clipping Weight[b] | 0.02 | <0.0001 | 0.001 |
| Surface Firmness | 0.003 | 0.007 | 0.002 |
| Ball Roll Distance | 0.007 | 0.01 | 0.12 |
| AUDPC[a] | 0.002 | \ | \ |
| **Field Trial 2** | | | |
| | *P value* | | |
| *Parameter* | *Treatment* | *Day* | *Treatment × Day* |
| Green Coverage | <0.0001 | <0.0001 | <0.0001 |
| NDVI | 0.78 | <0.0001 | 0.007 |
| Disease Severity | 0.002 | <0.0001 | <0.0001 |
| Chlorophyll Content | 0.004 | <0.0001 | 0.38 |
| Shoot Density | <0.0001 | <0.0001 | 0.29 |
| Surface Firmness | 0.01 | <0.0001 | 0.007 |
| Ball Roll Distance | 0.0001 | <0.0001 | 0.93 |
| AUDPC | 0.002 | \ | \ |

[a]AUDPC, Area Under the Disease Progress Curve; NDVI, Normalized Difference Vegetation Index

[b]Treatment × Week for Clipping Weight

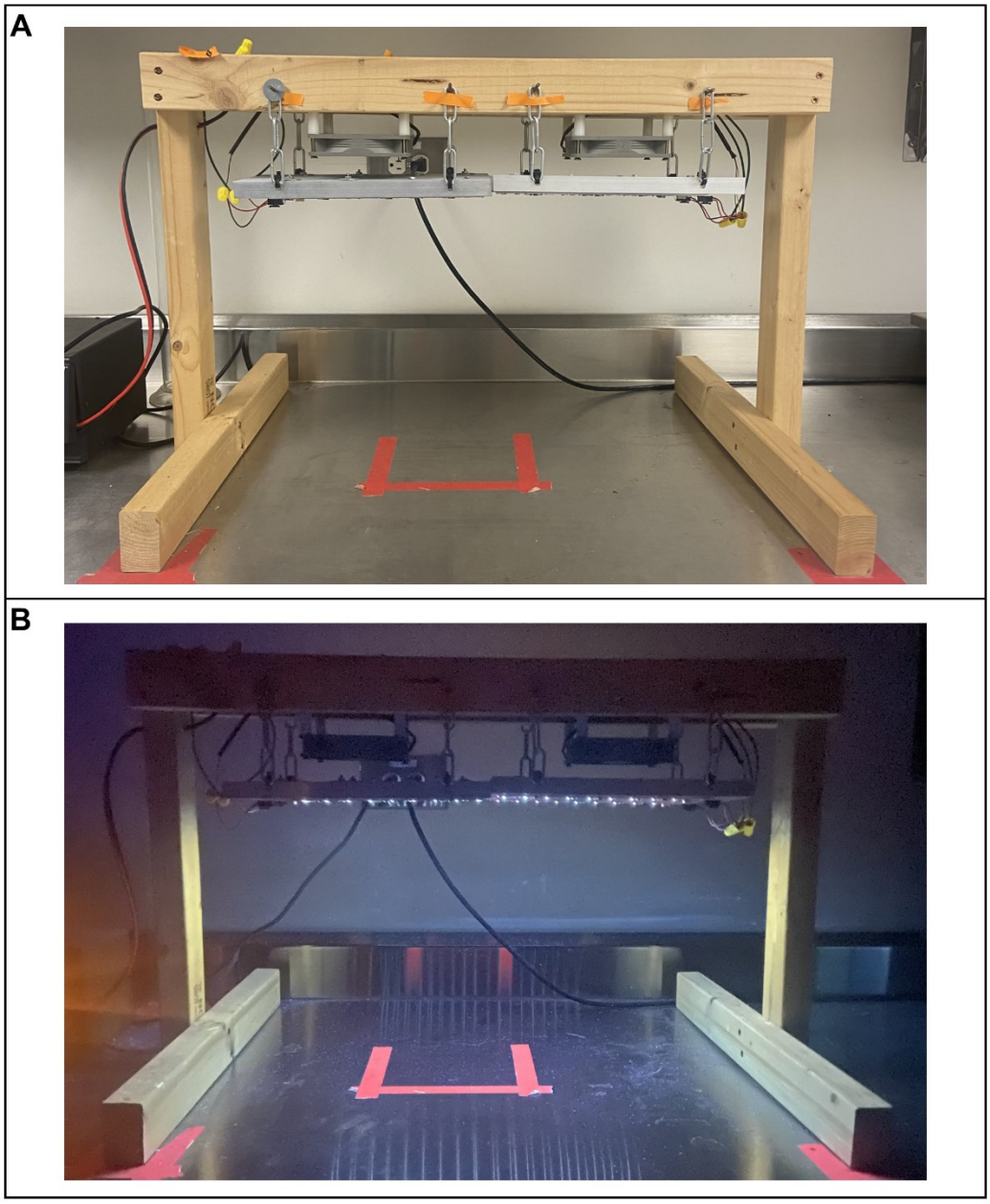


**Figure S3.1:** UV-C treatment setup used in *in vitro* and growth chamber trials, consisting of a UV-C lamp array adhered to heat sinks suspended from a wooden frame (A). Panel B shows the UV-C light powered on.


**Figure S3.2:** Modified Echo Robotics TM-1000 Turf Mower used to apply UV-C treatments in field trials (A). Panel B shows the UV-C light mounted to the bottom of the mower chassis, and Panel C shows nighttime operation in the field with the UV-C light powered on.

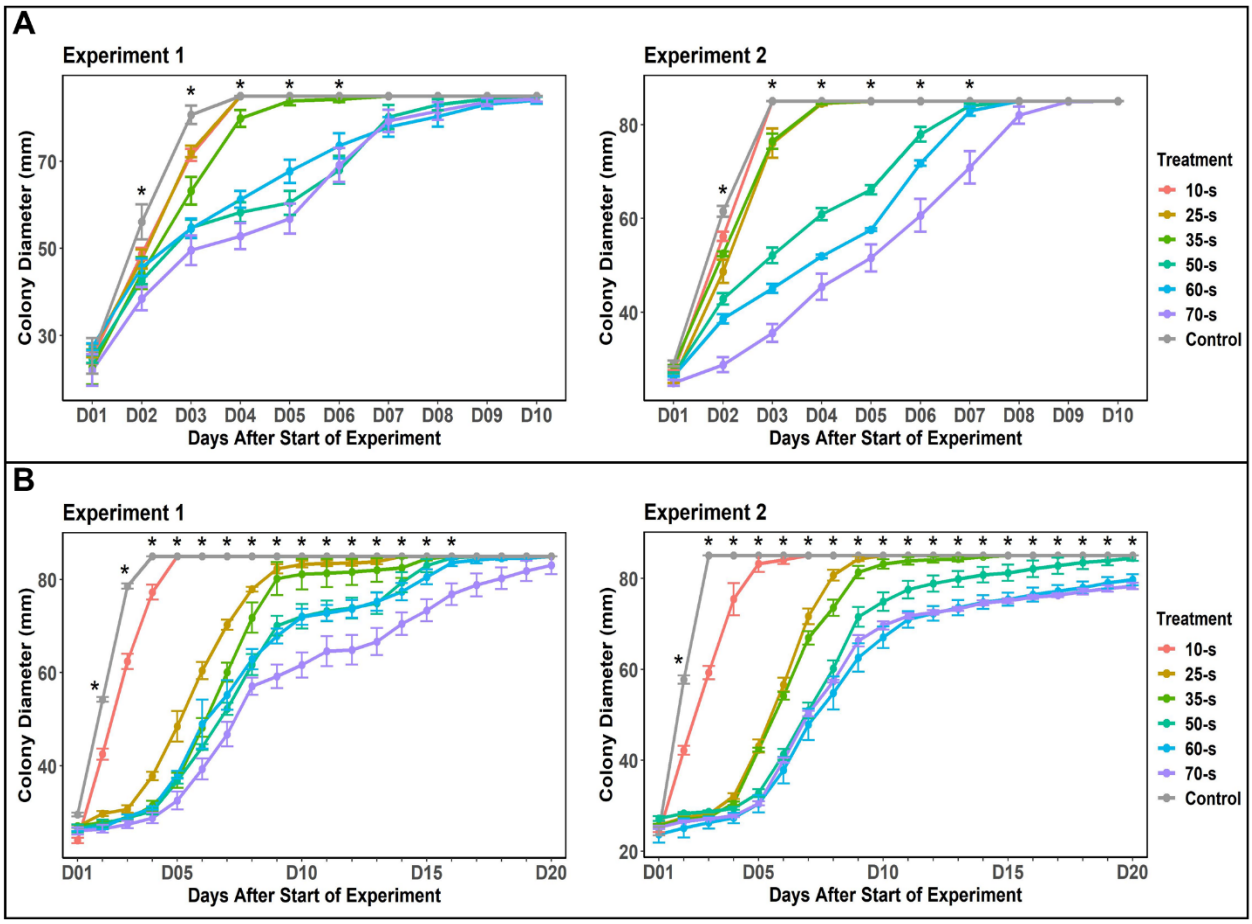


**Figure S3.3:** Mean colony diameter progression of *Clarireedia monteithiana* (DS8) fungal cultures treated with daily UV-C applications of 10-s, 25-s, 35-s, 50-s, 60-s, and 70-s, along with controls, in experiments 1 (left) and 2 (right) of *in vitro* trials 1 (A) and 2 (B). Error bars represent standard error. Statistical significance between treatments at each timepoint is indicated with '*' ($P = 0.05$, Tukey's HSD).

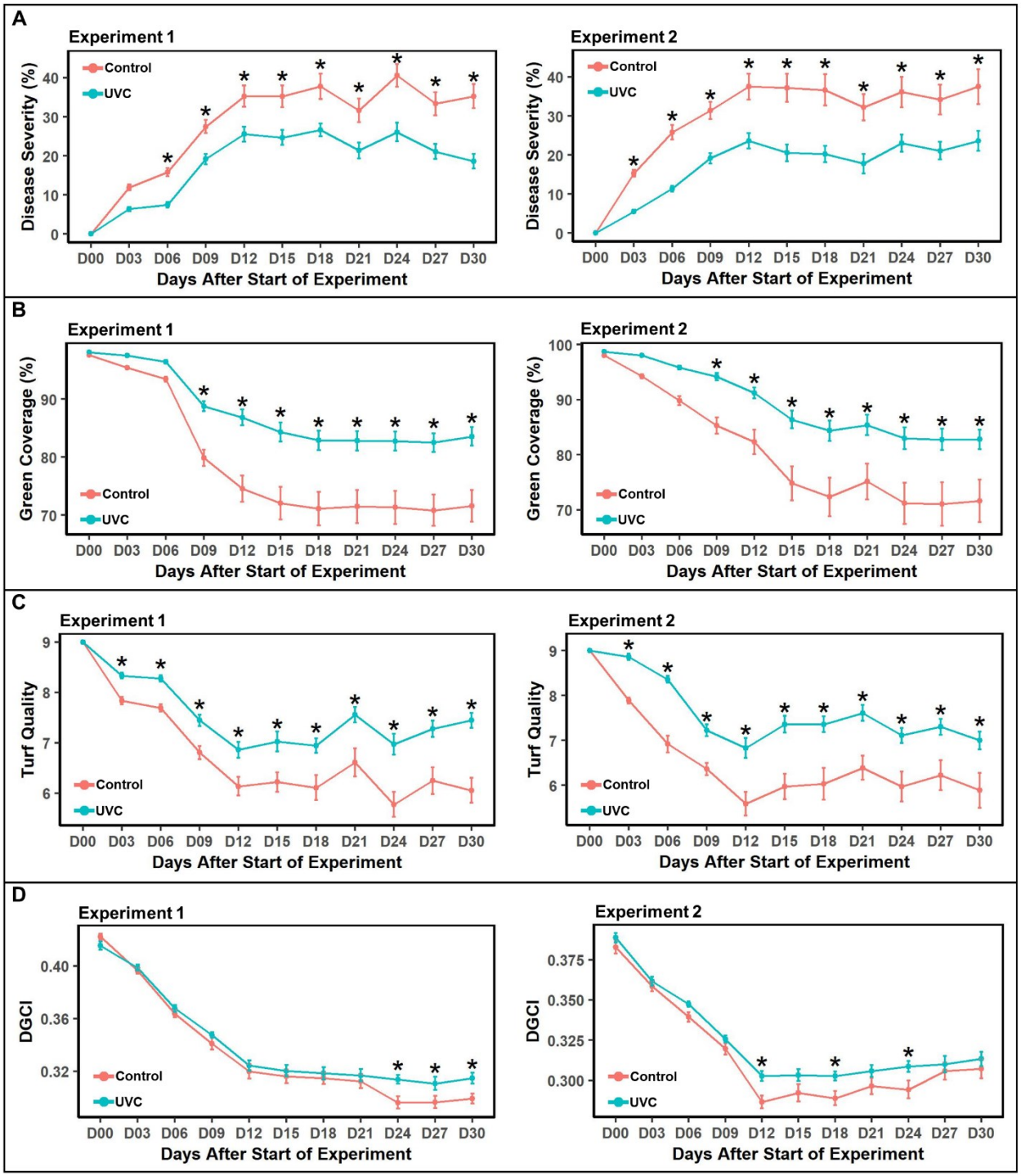


**Figure S3.4:** Mean disease severity (A), green coverage (B), visual turfgrass quality (C), and Dark Green Color Index (DGCI; D) progressions of dollar spot-inoculated seashore paspalum 'SeaStar' treated with daily 60-s UV-C applications versus a control in experiments 1 (left) and 2 (right) of Growth Chamber Trial 2. Error bars represent standard error. Statistical significance between treatments at each timepoint is indicated with '*' ($P = 0.05$, Tukey's HSD).

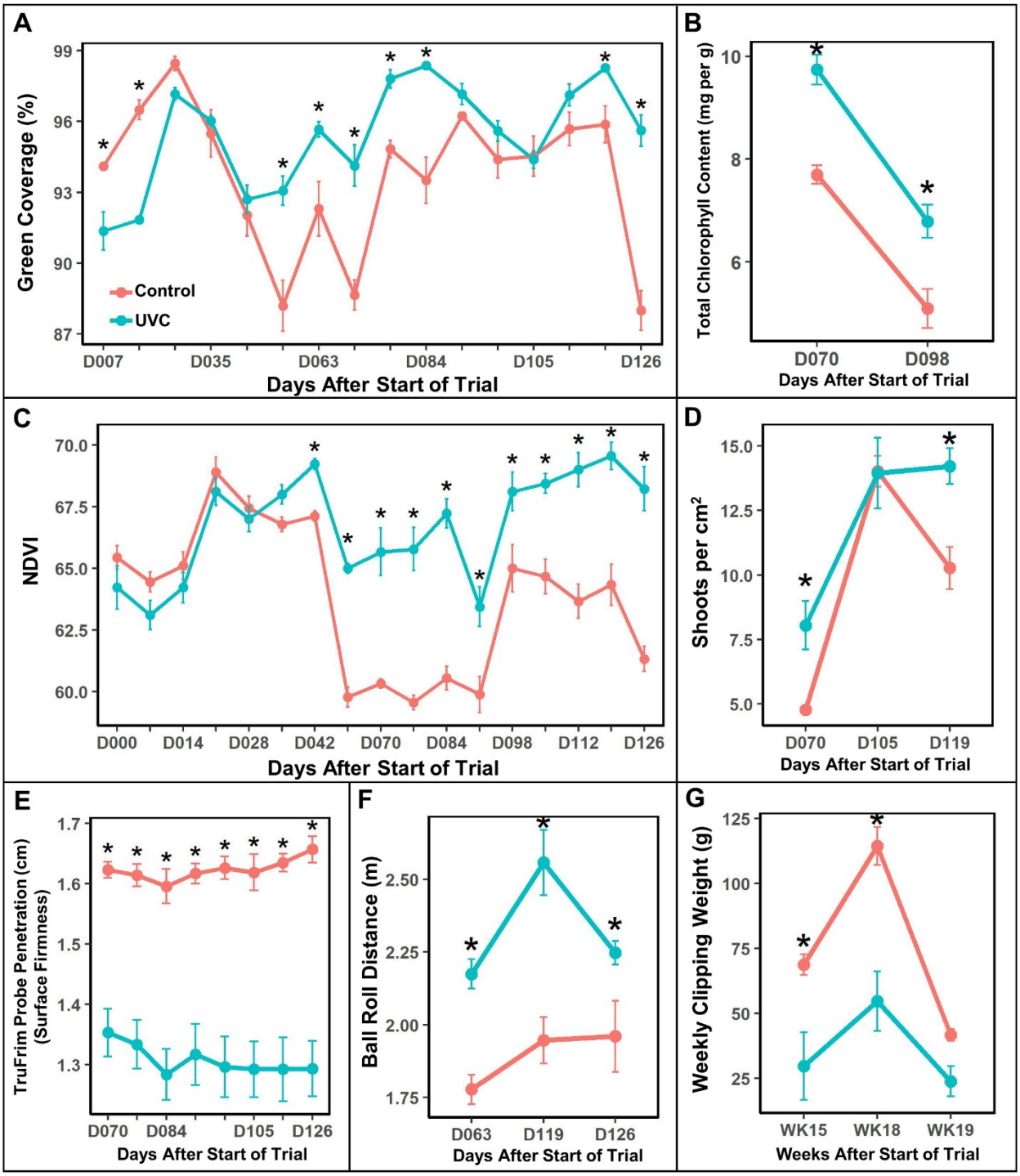


**Figure S3.5:** Mean green coverage (A), chlorophyll content (B), Normalized Difference Vegetation Index (NDVI; C), shoot density (D), probe penetration (surface firmness; E), ball roll distance (F), and weekly clipping weight (G) progressions of seashore paspalum 'SeaStar' treated with daily UV-C applications versus a control in Field Trial 1. Error bars represent standard error. Statistical significance between treatments at each timepoint is indicated with '*' ($P = 0.05$, Tukey's HSD).

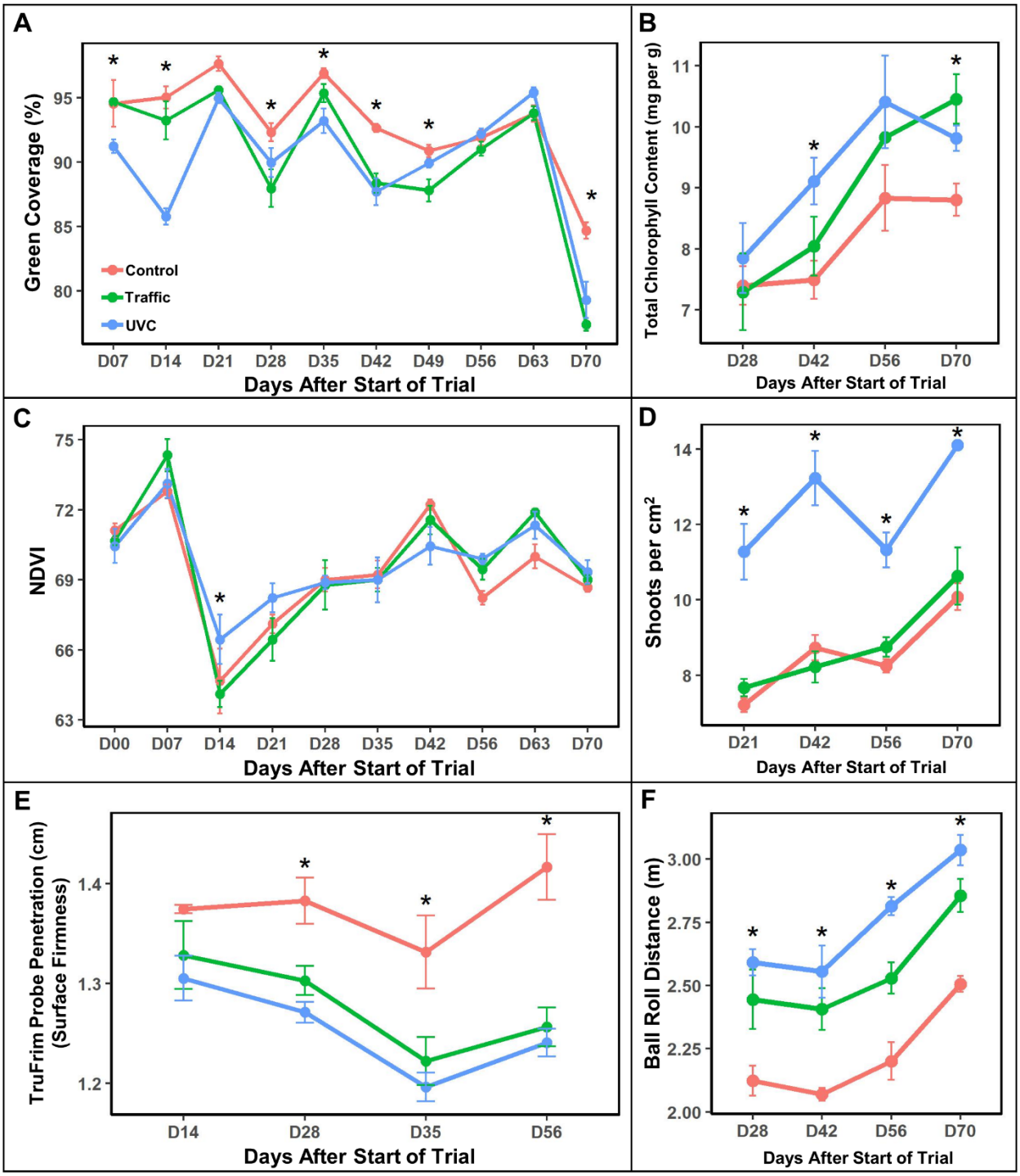


**Figure S3.6:** Mean green coverage (A), chlorophyll content (B), Normalized Difference Vegetation Index (NDVI; C), shoot density (D), probe penetration (surface firmness; E), and ball roll distance (F) progressions of seashore paspalum 'SeaStar' treated with daily UV-C applications versus control and traffic treatments in Field Trial 2. Error bars represent standard error. Statistical significance between treatments at each timepoint is indicated with '*' ($P$ = 0.05, Tukey's HSD).

# CHAPTER 4

# ASSESSING OXYGENATED AND OZONATED NANOBUBBLE WATER TREATMENTS FOR DOLLAR SPOT SUPPRESSION IN SEASHORE PASPALUM

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**Abstract**

Dollar spot (caused by *Clarireedia* spp.) is the most commonly occurring turfgrass disease in North America, and current disease control programs rely on frequent fungicide applications. The escalating occurrence of fungicide resistance in *Clarireedia* populations, coupled with stricter fungicide application restrictions, emphasizes the need for alternative management strategies. The use of oxygenated or ozonated water treatments has been effective as a plant disease management strategy. In field settings and in controlled environments, the impacts of oxygenated and ozonated nanobubble water treatments were evaluated for turf quality in seashore paspalum and their effectiveness in controlling dollar spot. Despite generating relatively high levels of dissolved oxygen (40 mg $L^{-1}$) or ozone (ca. 8 mg $L^{-1}$) in water treatments through nanobubble aeration, across all trials, these treatments did not cause damage to seashore paspalum tissues but were unsuccessful in controlling dollar spot. Additionally, tests comparing two different application methods (soil drench versus spray applications) for these treatments suggested that the application method used may not affect treatment efficacy. Overall, this study indicated that 1) oxygenated and ozonated nanobubble water treatments did not adversely affect seashore paspalum turf quality and 2) were ineffective in suppressing dollar spot in field and growth chamber settings.

**Keywords**: Dollar Spot, *Clarireedia*, Nanobubble, Turfgrass, Disease Management

## Introduction

Turfgrass is the most widely used groundcover in the United States, with an estimated 50 million acres spanning landscapes across the country (Milesi et al., 2005). Nationwide, the turfgrass industry is valued at over US$80 billion and supports more than 800,000 jobs (Chawla et al., 2018; Haydu et al., 2006). Dollar spot caused by *Clarireedia* spp. (Hu et al., 2019; Salgado-Salazar et al., 2018; Zhang et al., 2022) is one of the most detrimental diseases of turfgrass in the world (Vargas, 2018). It reduces playability and overall aesthetic value of both cool-season and warm-season turfgrasses by causing foliar blighting (Walsh et al., 1999).

Cultural controls can help mitigate dollar spot severity but often do not provide sufficient control on their own (Walsh et al., 1999). Additionally, highly resistant germplasm to dollar spot remains scarce for most turfgrass species (Sapkota et al., 2022). Therefore, the use of fungicides remains the most prominent and successful strategy for dollar spot management. However, multiple reports of dollar spot resistance across various locations in the United States have been documented for several major fungicide classes including the benzimidazoles (Ghimire et al., 2023; Warren et al., 1974), dicarboximides (Bishop et al., 2008; Detweiler et al., 1983), demethylation inhibitors (Golembiewski et al., 1995; Miller et al., 2002), and succinate dehydrogenase inhibitors (Popko et al., 2018; Sang et al., 2015). In addition, increasingly stringent environmental regulations on existing fungicides have left turfgrass practitioners with fewer available options to employ, emphasizing the need for alternative management strategies (Gullino and Kuijpers, 1994). Several physical control methods such as implementing oxygenated or ozonated water treatments could provide a viable and sustainable solution.

Oxygenated water refers to water that contains increased levels of dissolved oxygen ($DO_2$). Most of the early use and technological advancements in generating oxygenated water

(i.e. oxygenation or aeration) took place in the wastewater treatment industry, as evidenced by reports of wastewater aeration at an industrial scale dating back to the late 1800s (Boyle, 2003). Since then, the implementation of oxygenated water has expanded across several industries outside of wastewater, and recent studies have shown it can enhance a variety of physiological traits in plants. For example, in lettuce (*Lactuca sativa* L.) grown in lysimeters containing clayey or sandy soils, drip irrigation with oxygenated water (20 mg $L^{-1}$ $DO_2$) led to increased yield and decreased membrane leakage in roots and leaves, which consequently enhanced root viability and chlorophyll content (Baram et al., 2022). Similarly, compared to controls, oxygenated drip irrigation (6-9 mg $L^{-1}$ $DO_2$) in greenhouse cucumbers (*Cucumis sativus* L.) led to improved yields by enhancing traits such as leaf area index, net photosynthetic rate, and nutrient absorption (Ouyang et al., 2023). Furthermore, there have been a few cases documented in which oxygenated water decreased plant disease severity. In tomatoes (*Solanum lycopersicum* L.) grown in a hydroponic setting, plants that received oxygen treatments (5-7 mg $L^{-1}$ $DO_2$) via compressed air bubbling exhibited a two-fold increase in root and shoot growth compared to non-aerated treatments, as well as a significant reduction in colonization of roots by *Pythium* spp. (Chérif et al., 1997). Similarly, Fraedrich and Tainter (1989) evaluated the effects of low (0-1 mg $L^{-1}$ $DO_2$) and high (6.6-7.4 mg $L^{-1}$ $DO_2$) dissolved oxygen levels on *Phytophthora cinnamomi* infection in shortleaf (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) grown in holding tanks. Across four separate experiments, plants grown in high oxygen conditions consistently exhibited significantly lower susceptibility to the pathogen (Fraedrich and Tainter, 1989).

Ozone is a powerful oxidant that can be used to kill microorganisms such as viruses, bacteria, and fungi (Korzun et al., 2008) and has been used for various disinfection purposes since the early 1900s (Gomella, 1972). Its official sanctioning for use as a food sanitizer in 1997

(Sopher et al., 2002) prompted a surge in research efforts exploring applications of ozonated water (i.e. water that contains increased levels of dissolved ozone ($DO_3$)) across several food-related fields (Sarron et al., 2021). In the agricultural field, several studies have shown that ozonated water treatments can have a direct antagonistic effect against plant pathogens. Fujiwara and Fujii (2002) found that ozonated water spray treatments (4 mg $L^{-1}$ $DO_3$) suppressed the spread of powdery mildew (*Sphaerotheca fuliginea*) in greenhouse cucumber for up to 14 days compared to non-treated controls and did not cause visible injury to plant tissues. Veronico et al. (2017) found that ozonated water soil drenches (10 mg $L^{-1}$ $DO_3$) significantly reduced nematode (*Meloidogyne incognita*) infection rate in tomatoes grown in a growth chamber by 23% compared to controls. Sprayed ozone foliar treatments (10 mg $L^{-1}$ $DO_3$) also led to a reduction in tomato spotted wilt virus severity in growth chamber tomato, as treated plants displayed 20% less disease than controls (Prigigallo et al., 2019). Moreover, using conidial suspensions mixed with aqueous ozone, He et al. (2015) found that *in vitro* ozonated water treatments (1.6-1.8 mg $L^{-1}$ $DO_3$) caused a 3.3 log reduction in *Alternaria solani* conidia production compared to controls, as well as a 3.7 to 5.0 log reduction in *Cladosporium fulvum* conidia production.

Traditional methods of generating oxygenated or ozonated water treatments typically involve the use of bubble diffusers, mixing pumps, or venturi air injectors (Zainuddin et al., 2017). However, since the discovery of nanobubbles in 1994 (Parker et al., 1994), there has been an expansion in research activities exploring the use of nanobubble aeration to produce these treatments. Nanobubbles are gas-filled cavities typically less than 5 μm in size (Khan et al., 2020). Their small size makes them less buoyant, enabling them to remain suspended in water for longer periods of time (Agarwal et al., 2011). Additionally, the negative surface charge of nanobubbles contributes to their prolonged stability in water (Senthilkumar et al., 2018). High

internal pressure within these tiny bubbles also enables the gases they contain to quickly dissolve into surrounding liquid (Eriksson and Ljunggren, 1999). Taken together, these unique properties of nanobubbles render them highly effective at discharging gas into liquids (Agarwal et al., 2011).

Despite the benefits that oxygenated or ozonated water treatments impart to various cropping systems, information on their effectiveness in turfgrass health and disease management is still limited. The goals of this study were to 1) assess the impacts on turf health resulting from repeated applications of oxygenated and ozonated nanobubble water, and 2) investigate the effectiveness of these treatments against dollar spot in both field and growth chamber settings.

## Materials and methods

### Plant material

Seashore paspalum (*Paspalum vaginatum* Sw.) 'Sea Isle 1' was used in all growth chamber and field trials. For growth chamber experiments, 7.6 cm x 7.6 cm turfgrass plugs were extracted from a sward of 'Sea Isle 1' located at the University of Georgia, Griffin Campus, Griffin, GA. After extraction, native soil was cut away from grass plugs to leave at least 3 cm of intact roots, and they were planted in 7.6 cm x 7.6 cm Kord nursery pots (HC Companies, OH, USA) filled with potting mix (Metro Mix 852; Sun Gro Horticulture, MA, USA). After planting, plugs were left in a greenhouse (24-32˚C, 78% relative humidity) to establish for at least one month. During establishment, all plants received a weekly fertilization treatment of a mix of water and 0.7 g L MiracleGro Water Soluble All-Purpose Plant Food (NPK 24-8-16) (The Scotts Company LLC, USA), as per manufacturer's guidelines. Plants were routinely trimmed to maintain a canopy height of 2.5 cm. Additionally, plants were frequently monitored and culled to

ensure the use of high quality, disease-free plant material in growth chamber trials. A week prior to trial initiations, plants were flushed with water to remove any residual fertilizer that may have influenced dollar spot incidence or severity (Steketee et al., 2016).

The field from which turfgrass plugs were taken for growth chamber experiments was used in field trials. The field was comprised of seashore paspalum 'Sea Isle 1'. During field trials, grass was mowed on a weekly basis at a height of 2 cm, and irrigation ran to supply the field with 2.5 cm of water per week, including rainfall. No fertilizer or pesticide applications were made to the field during trials, and weeds were removed manually as needed.

**Fungal material and inoculum preparation**

A dollar spot isolate (DS8) collected in 2019 from a seashore paspalum field located at the University of Georgia, Griffin Campus, Griffin, GA was utilized in all field and growth chamber trials that required artificial inoculation. The isolate was identified by Sapkota et al. (2020) as *C. monteithiana* based on species-specific SNPs within the internal transcribed spacer (ITS) region (ITS sequence stored in NCBI GenBank under 'MT497854') (Salgado-Salazar et al., 2018). The isolate was stored in a sterile grain mixture of oat, barley, and wheat at -20˚C and in a Microbank vial (Pro-Lab Diagnostics, ON, Canada) at -80˚C.

Fresh DS8 inoculum was prepared prior to the start of each field or growth chamber trial and was created following protocols from Steketee et al. (2016). Briefly, a seed of DS8 storage grain was plated onto potato dextrose agar (PDA) media and grew for 7 days at room temperature under 12-hour light. While the isolate grew, a 1000 ml Erlenmeyer flask was filled with an approximate 200 ml by volume equal mixture of oat, barley, and wheat, and 250 ml of sterile water was added to the grain mixture. The flask was sealed and covered, and the grain

mixture was left to soak overnight. The flask containing the grain mixture was then autoclaved once a day for the next two days. Afterward, 2 $cm^2$ squares of DS8-infested PDA media were cut out under sterile conditions and added to the flask to create the inoculum. The inoculum flask was shaken once daily to promote even proliferation of the pathogen throughout the grain mixture. After two weeks, the inoculum was ready to be used in field or growth chamber trials.

**Oxygenated and ozonated nanobubble water preparations**

Oxygenated and ozonated nanobubble water were generated by subjecting tap water (TW) to nanobubble aeration via the N-5 Nanobubble Aeration Unit, which is developed and owned by the NABAS Group (NABAS Group Inc., Rockville, MD) as a proprietary technology. Oxygenated nanobubble water (OXN) and ozonated nanobubble water (OZN) were generated with the N-5 according to the manufacturer's instructions, and during generation, a Hanna HI98198 Optical Dissolved Oxygen Meter (Hanna Instruments, USA) was used to continuously monitor water quality parameters, such as temperature and dissolved oxygen concentration. After producing OXN and OZN, each respective water type was collected in sterile reservoirs that were compatible with sprayers used in field or growth chamber trials. In all trials, OZN was applied at a $DO_3$ concentration of ca. 8 mg $L^{-1}$, and OXN was applied at a $DO_2$ concentration of 40 mg $L^{-1}$. All applications of OXN and OZN were made promptly (within 30 minutes) following nanobubble water generation. Tap water used for control treatments in trials and for generating nanobubble water treatments was sourced from the University of Georgia, Griffin Campus, Griffin, GA. $DO_2$ concentrations of the tap water ranged from 6.5-8.0 mg $L^{-1}$.

## Growth chamber nanobubble water experiments

Three *in planta* growth chamber trials were carried out to test the effects of oxygenated and ozonated nanobubble water on turfgrass tissues and for dollar spot suppression. The initial trial, Growth Chamber Trial 1 (GCT1), was conducted to evaluate whether nanobubble water treatments had any phytotoxic effects on turfgrass tissues in the absence of disease, as well as to establish a treatment application interval to be used in subsequent growth chamber and field trials. A second growth chamber trial, Growth Chamber Trial 2 (GCT2), was conducted to test the efficacy of nanobubble water treatments in suppressing dollar spot. The final growth chamber trial, Growth Chamber Trial 3 (GCT3), was implemented to evaluate dollar spot suppression from different methods of nanobubble water application, namely soil drench versus spray applications.

### *Phytotoxicity and application scheduling for nanobubble water treatments (GCT1)*

Seashore paspalum 'Sea Isle 1' pots were acquired and maintained as previously described. Three different water treatments were utilized in this trial, and each water treatment was sprayed at three different application intervals for a total of nine treatments. The nine treatments were: 1) TW sprayed every day, 2) TW sprayed every other day, 3) TW sprayed every 3 days, 4) OXN sprayed every day, 5) OXN sprayed every other day, 6) OXN sprayed every 3 days, 7) OZN sprayed every day, 8) OZN sprayed every other day, and 9) OZN sprayed every 3 days. The trial was laid out as a completely randomized design (CRD) with 4 replicated pots per treatment. All treatments were applied using a Generation 3 Research Track Sprayer (DeVries Manufacturing Inc., MN, USA) with a TeeJet XR8002 flat fan nozzle (TeeJet Technologies, USA) at 276 kPa, and pots were sprayed until runoff occurred. Throughout the trial, pots were kept in a Percival Scientific E-41L2 growth chamber (Percival Scientific, IA, USA) set at day

and night temperatures of 25˚C and 16˚C, respectively, 100% relative humidity, and a 12-hour photoperiod. The trial lasted 33 days.

***Efficacy of nanobubble water treatments against dollar spot (GCT2)***

Based on results from GCT1, in GCT2, each water treatment (OXN, OZN, and TW) was applied every 3 days. Similarly, seashore paspalum 'Sea Isle 1' was also utilized in this trial. The same sprayer, nozzle, and sprayer settings used in GCT1 were used in GCT2, as well as the same growth chamber and growth chamber settings. The trial was laid out as a CRD with 10 replicated pots per treatment. All pots in this trial were artificially inoculated with DS8-infested grain (*C. monteithiana*), and treatments started on the same day plants were inoculated. For inoculation, five seeds of grain inoculum were introduced to the foliar canopy of each pot, and black plastic bags were used to cover pots for the first 6 days of the trial to create high humidity conditions that favored dollar spot infection. Overall, the trial lasted 33 days and was repeated (experiments one and two).

***Efficacy of nanobubble water treatments against dollar spot using two application methods (GCT3)***

Seashore paspalum 'Sea Isle 1' pots were utilized in this trial, and all pots were inoculated with DS8-infested grain in the same way as in GCT2. Treatments started the same day pots were inoculated, and treatments in this trial included: 1) TW spray (TWs), 2) TW drench (TWd), 3) OXN spray (OXNs), 4) OXN drench (OXNd), 5) OZN spray (OZNs), and 6) OZN drench (OZNd). All treatments were applied every 3 days. Spray treatments were applied in the same way as in GCT1 and GCT2, and soil drench treatments were applied by pouring 50 ml of the respective treatment into each corresponding pot. The trial was set up as a CRD with 6

replicated pots per treatment with the same growth chamber settings used in GCT1 and GCT2. The trial lasted 33 days and was repeated (experiments one and two).

**Field nanobubble water experiments**

Two field trials were conducted to explore the impacts of oxygenated and ozonated nanobubble water on the health of turf tissues and in the suppression of dollar spot. Field Trial 1 (FT1) was carried out to evaluate any potential phytotoxic effects nanobubble water treatments could cause to turfgrass tissues in the absence of disease. Field Trial 2 (FT2) was conducted in parallel to FT1 in a different area of the same field, and the goal of FT2 was to evaluate the impact nanobubble water treatments had on dollar spot.

***Phytotoxicity of nanobubble water treatments in the field (FT1)***

Because the objective of FT1 was to evaluate potential phytotoxic effects nanobubble water treatments could cause to turf in the absence of disease, plots were treated with a biweekly rotation of propiconazole [1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole] (Banner Maxx; Syngenta, USA) and fluxapyroxad [3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide] (Xzemplar; BASF, USA) to prevent disease. The trial involved three treatments: 1) OXN, 2) OZN, and 3) TW and was laid out as a randomized complete block design with 5 replications per treatment. Each individual field plot measured 0.60 m x 0.91 m. 500 ml of each treatment was applied every 3 days to each corresponding plot using a handheld $CO_2$-pressurized boom sprayer with a TeeJet XR8002 flat fan nozzle at 276 kPa. A spray shield was utilized during treatment applications to prevent off-target drift. The trial started in the spring of 2022 and lasted a total of 9 weeks (April 22-June 24); it was repeated in the fall (August 25-October 27).

### *Efficacy of nanobubble water treatments against dollar spot in the field (FT2)*

For FT2, trial design, treatments, and plot dimensions matched those of FT1, as well as procedures for treatment applications. However, because the goal of this trial was to evaluate the efficacy of nanobubble water treatments against dollar spot, plots were not treated with fungicides and were artificially inoculated with DS8-infested grain (*C. monteithiana*). Inoculation took place 4 weeks after trial initiation and was executed by uniformly spreading 18 g of DS8 inoculum to each plot. FT2 started in the spring of 2022 and lasted a total of 9 weeks (April 22-June 24); it was repeated in the fall (August 25-October 27).

## Rating procedures and data analysis

Several different parameters related to turfgrass quality and, where applicable, disease severity were recorded across trials. Visual turf quality, green coverage, and Dark Green Color Index (DGCI) ratings were taken in GCT1 and FT1. Green coverage is a measurement of ground or pot percentage that is covered by green turf, and DGCI is a measurement of turf color. The same ratings were taken in GCT2, GCT3, and FT2, along with scoring for visual disease severity and area under the disease progress curve (AUDPC).

Scoring for visual turf quality encompassed factors such as turfgrass density, uniformity, and color. Rating was done via the National Turfgrass Evaluation Program 1 to 9 quality rating scale, where 1 = completely dead turfgrass, 6 = minimally acceptable turfgrass, and 9 = dark green dense turfgrass (Morris and Shearman, 2008). Disease severity scoring was performed by visually determining the percent plot or pot area blighted by dollar spot (0-100% linear scale, where 0= turf area entirely asymptomatic and 100= turf area entirely symptomatic) (Putman and Kaminski, 2011). To avoid interpersonal variation, both visual turf quality and disease severity

ratings were always taken by the same person. Digital imaging analysis for green coverage and DGCI was done on the same days as visual scoring by taking pictures of plots or pots using a Canon EOS Digital Rebel XT camera (Canon Inc., NY, USA) mounted to a lightbox. The lightbox was implemented to maintain consistent lighting conditions while taking pictures and was constructed with insulation foam and six LED lights. Field Analyzer software (https://www.turfanalyzer.com/field-analyzer) (Field Analyzer, AR, USA) was used to analyze images. Default settings were used for DGCI, and settings for green coverage analysis included low hue of 35 to 45, high hue of 360, low saturation of 15 to 20, high saturation of 100, low brightness of 0, and high brightness of 90.

All scoring in all trials started on D00, the initiation day of each trial, except in GCT2 and GCT3. Scoring in these two trials started 6 days post-inoculation (D06) to allow adequate time for dollar spot infection to occur. For field trials, all plots were rated on a weekly basis, and for growth chamber trials, all pots were rated every 3 days. All trial data were analyzed using R statistical software (v.4.3.2). Data were subjected to repeated measures ANOVAs and means were separated using Tukey's HSD test ($p = 0.05$). All figures were created using various functions in *ggplot2*, *ggforce*, and *ggpubr* packages. Blight percentages from disease severity scoring were used to calculate AUDPC (Madden et al., 2007) according to the following formula:

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where $y_i$ is an assessment of disease severity (% turfgrass area blighted) at the $i^{th}$ observation, $t_i$ is time (day) at the $i^{th}$ observation, and $n$ is the total number of observations.

## Results

### Phytotoxicity and application scheduling for nanobubble water treatments (GCT1)

Nine different treatments (3 water treatments × 3 application intervals) were implemented in this growth chamber trial to test whether oxygenated or ozonated nanobubble water caused damage to turf tissues in the absence of disease. The mean turf quality, green coverage, and DGCI scores for all pots over the course of the entire trial were 8.9, 96.6%, and 0.452, respectively. No significant differences ($p>0.05$) were observed among any of the nine treatments for any of these metrics (Table 4.1). Furthermore, analysis conducted by scoring day for each parameter also revealed no significant differences ($p>0.05$) among the treatments. Taken together, these results indicate that nanobubble water treatments sprayed at any application interval tested over 33 days did not cause damage to turf tissues. In light of the outcomes from this initial study, an application interval of every 3 days was chosen for subsequent growth chamber and field trials.

### Efficacy of nanobubble water treatments against dollar spot (GCT2)

The effects of three water treatments (OXN, OZN, TW) on turf quality, green coverage, DGCI, AUDPC, and disease severity were evaluated at ten distinct timepoints over 33 days. This assessment was conducted within a growth chamber across duplicate experiments (experiments one and two). The effect of 'experiment' was statistically significant ($p<0.05$) for all five parameters assessed. Therefore, data from the two experiments were subjected to separate statistical analyses.

In experiment one, mean turf quality scores across the entire experiment were similar, with OZN at 6.4, TW at 6.3, and OXN at 6.1. Treatment TW (80.9%) had the highest overall

mean green coverage score, followed by OZN (78.2%) and OXN (78.1%) treatments, respectively. Mean DGCI scores across all timepoints were 0.417, 0.415, and 0.413 for treatments OZN, OXN, and TW, respectively. In terms of dollar spot infection, OZN (25.5%) exhibited the lowest mean disease severity compared to TW (25.9%) and OXN (28.7%), and mean AUDPC values showed a similar trend (OZN: 693, TW: 706, OXN: 774). Analysis conducted across the entire experiment revealed no significant differences ($p>0.05$) among the three treatments for any of the five parameters assessed (Figure S4.1). Analysis for each parameter by each timepoint revealed one statistically significant result. This occurred on D33 when the mean turf quality score of OXN was significantly lower ($p<0.05$) than TW but not OZN (Figure 4.1).

In experiment two, mean turf quality scores (OXN: 4.6, TW: 4.3, OZN: 4.2) for treatments across all timepoints mirrored green coverage (OXN: 60.6%, TW: 58.0%, OZN: 55.6%) and DGCI (OXN: 0.344, TW: 0.335, OZN: 0.332) scores. Regarding disease severity, OXN treatments exhibited the lowest mean score across the entire experiment (55.0%), followed by TW (58.2%) and OZN (59.5%) treatments, respectively. Trends in AUDPC scoring were similar (OXN: 1516, TW: 1608, OZN: 1640). Like experiment one, no significant differences ($p>0.05$) were observed among the three treatments for all parameters assessed across the entire experiment (Figure S4.1), nor were there any differences ($p>0.05$) noted for each individual scoring day.

**Efficacy of nanobubble water treatments against dollar spot using two application methods (GCT3)**

The effects of six treatments (3 water treatments x 2 application methods) on turf quality, green coverage, DGCI, AUDPC, and disease severity were assessed across ten distinct timepoints over 33 days. This evaluation was carried out in a growth chamber, spanning two replicate experiments (experiments one and two). The effects of 'experiment' and 'experiment x treatment' were statistically non-significant ($p>0.05$) for turf quality, green coverage, AUDPC, and disease severity parameters. Therefore, data from these four metrics across the two experiments were subjected to combined statistical analyses. Regarding DGCI, the effect of 'experiment' was statistically significant ($p<0.05$). Therefore, it was subjected to separate statistical analysis for each experiment. Furthermore, the effect of 'application method' was found to be statistically insignificant ($p>0.05$) in all analyses conducted for GCT3. Given this result, further testing of different application methods was discontinued in field trials.

Across the entire trial, OZNd treatments exhibited the highest mean turf quality (6.8) and green coverage (77.2%) scores and the lowest mean disease severity (24.5%) and AUDPC (652) scores. Conversely, OXNd treatments exhibited the lowest mean turf quality (6.0) and green coverage scores (65.2%) and the highest mean disease severity (35.0%) and AUDPC (926) scores. Statistically, all six treatments were observed to be similar to each other in terms of turf quality ($p>0.05$), green coverage ($p>0.05$), disease severity ($p>0.05$) and AUDPC ($p>0.05$), across the entire trial (Figure S4.2) and at each timepoint analyzed.

Regarding DGCI, OXNs had the highest overall mean score in experiment one (0.347) and TWd had the lowest (0.333). Conversely, in experiment two, TWs garnered the highest mean score (0.331) and OXNd the lowest (0.312). In both experiments, all six treatments were found

to be statistically similar ($p>0.05$) to each other in terms of overall mean DGCI. Analysis conducted at each timepoint for each experiment revealed statistically significant differences ($p<0.05$) among certain treatments on days D9, D12, D21, D30, and D33 in experiment one and on days D24, D27, D30, and D33 in experiment two (Figure 4.2). Differences noted at specific timepoints in experiment one did not follow any discernible trends or patterns across treatments. In experiment two, OXNd and OXNs treatments exhibited lower DGCI towards the end of the experiment, contributing to the significant results recorded in the final 4 days.

**Phytotoxicity of nanobubble water treatments in the field (FT1)**

Duplicate experiments were conducted in the field to evaluate the effects of three different water treatments (OXN, OZN, and TW) on turf quality, green coverage, and DGCI under conditions where disease was not present. One experiment was held in the spring of 2022 and the other in the fall. In both experiments, data was collected across ten timepoints over 63 days. The effects of 'experiment' and 'experiment x treatment' were statistically insignificant ($p>0.05$) for turf quality, so it was subjected to combined statistical analysis for the two experiments. The effect of 'experiment' was statistically significant ($p<0.05$) for green coverage and DGCI. Therefore, these parameters were subjected to separate statistical analyses for spring and fall experiments.

In terms of overall visual turf quality, mean scores for all treatments were similar (OXN: 8.4, OZN: 8.4, TW: 8.5). There were no statistically significant differences ($p>0.05$) found among the three treatments across the entire trial, nor were there any differences ($p>0.05$) noted for each of the ten scoring days.

In terms of green coverage, OXN treatments garnered the highest overall mean score in the spring experiment (94.1%), followed by OZN (94.0%) and TW (92.6%) treatments, respectively. In the fall experiment, TW finished as the top scorer (94.8%), followed by OXN (94.5%) and OZN (94.4%), respectively. Statistically, there were no significant differences ($p>0.05$) observed for overall mean green coverage among any of the treatments in both fall and spring experiments. Similarly, for each timepoint analyzed in the fall experiment, there were no significant differences ($p>0.05$) observed for green coverage among all treatments. However, in the spring experiment, there were a few rating dates where treatments significantly differed from one another. On D00, green coverage in TW plots was significantly lower ($p<0.05$) than green coverage in OXN and OZN plots. A similar phenomenon also occurred on D07, where green coverage in TW plots was significantly lower ($p<0.05$) than OXN plots, but not OZN plots (Figure 4.3). Since these differences in green coverage both occurred within the first week of the spring experiment, including the first day, they are likely attributed to the inherent variability of grass growth in the field rather than to the treatments themselves. This is further supported by the spring experiment coinciding with a portion of the typical spring 'green up' period for warm-season grasses in Georgia, where grasses are transitioning out of dormancy into active growth.

Overall mean DGCI scores in the spring experiment ranged from 0.443-0.449 (TW-OXN) and from 0.368-0.376 (OZN-TW) in the fall. DGCI metrics followed trends similar to those of turf quality and green coverage in that there were no significant differences ($p>0.05$) found among treatments across spring or fall experiments. Furthermore, of the 300 data points gathered for DGCI across the two experiments, only one statistically significant result was recorded. This happened on D56 of the fall experiment when DGCI scores of OZN plots were significantly lower ($p<0.05$) than those of both OXN and TW plots (Figure 4.4).

**Efficacy of nanobubble water treatments against dollar spot in the field (FT2)**

To test the capability of nanobubble water treatments in mitigating dollar spot, a field trial was conducted, under artificial inoculation, to evaluate the effects of OXN, OZN, and TW treatments on turf quality, green coverage, DGCI, disease severity, and AUDPC. The field trial was performed over 63 days and was replicated across two different seasons (spring and fall of 2022). The effects of 'experiment' and 'experiment x treatment' were statistically insignificant ($p>0.05$) for turf quality, green coverage, disease severity, and AUDPC, so these parameters were subjected to combined statistical analysis for the two experiments. DGCI was subjected to separate statistical analysis for the two experiments due to a significant effect ($p<0.05$) of 'experiment'.

In terms of overall mean turf quality and green coverage, OZN scored highest in both categories (6.8 turf quality, 80.9% green coverage), followed by OXN (6.7 turf quality, 80.8% green coverage) and TW (6.6 turf quality, 80.0% green coverage). Overall mean disease severity and AUDPC was lowest in OXN plots (21.0% disease, 1149 AUDPC), followed by TW (21.5% disease, 1204 AUDPC) and OZN (22.2% disease, 1248 AUDPC) plots, respectively. Differences in turf quality, green coverage, disease severity, and AUDPC among the three treatments were not significant ($p>0.05$) across the entire trial (Figure S4.3) or for each timepoint analyzed ($p>0.05$).

Regarding DGCI, in both spring and fall experiments, TW exhibited the highest overall mean score (0.420 spring, 0.354 fall), followed by OZN (0.419 spring, 0.353 fall) and OXN (0.414 spring, 0.349 fall), respectively. However, no significant differences ($p>0.05$) in overall mean DGCI were observed among the treatments in either experiment. Among all the data collected from both experiments, only one statistically significant DGCI result was recorded,

which occurred on D21 of the spring experiment. On this day, mean DGCI scores for OXN were significantly lower ($p<0.05$) than those of both OZN and TW (Figure 4.5).

**Discussion**

In this study, we investigated the effects of oxygenated and ozonated water treatments on dollar spot development and turfgrass quality in seashore paspalum. Across all field and growth chamber trials conducted, we found that these treatments did not cause any noticeable phytotoxic damage to seashore paspalum tissues but failed to mitigate dollar spot severity in any capacity. These findings are consistent with some previous studies but conflict with others. For example, using the same application interval implemented in most of our trials (every 3 days), Fujiwara and Fujii (2002) found that ozonated water spray treatments (4 mg $L^{-1}$ $DO_3$) contained the spread of powdery mildew in cucumber and did not cause any visible damage to plants. The contrasting results between our study and theirs could be due to different mechanisms of pathogen spread and/or infection. Most powdery mildews spread superficially on plant surfaces, so pathogens in their study were likely directly exposed to ozone treatments. Systemic infection that occurs with dollar spot could have limited direct pathogen exposure to nanobubble water treatments in our trials (Allen et al., 2005). Moreover, Fraedrich and Tainter (1989) found that shortleaf and loblolly pine grown in high oxygen conditions were less susceptible to *Phytophthora cinnamomi* infection compared to plants grown in low oxygen conditions. However, $DO_2$ concentrations in their low oxygen treatments ranged from 0-0.25 mg $L^{-1}$, which essentially created an anaerobic environment. Rather than attributing low $DO_2$ levels directly to increased pathogen viability, they suggested that anerobic conditions predisposed plant roots to pathogen infection. In our experiments, we did not include treatments that subjected plants to such anaerobic environments.

Furthermore, mirroring results from our trials, several studies have noted the limited effectiveness of oxygenated or ozonated water treatments in suppressing plant disease. Using the same nanobubble aeration unit as in our trial, Díaz-Pérez et al. (2023) recently found that oxygenated (1.9 mg $L^{-1}$ $DO_2$) and ozonated (ca. 8 mg $L^{-1}$ $DO_3$) drip irrigation did not affect field tomato growth, fruit yield, or the incidence of tomato yellow leaf curl and southern blight (*Sclerotium rolfsii*). Similarly, over the course of two growing seasons, McDaniel et al. (2024) found that ozonated water spray treatments (ca. 1 mg $L^{-1}$ $DO_3$) failed to provide any meaningful control of grapevine powdery mildew (*Erysiphe necator*) in field grapes (*Vitis vinifera* L.) across two different vineyard locations. In strawberry (*Fragaria* × *ananassa* Duch.) field trials, Moor et al. (2023) reported higher incidence of botrytis fruit rot (*Botrytis cinera*) occurring from ozonated water spray treatments (2 mg $L^{-1}$ $DO_3$) compared to control treatments. The authors attributed the increase in disease incidence to ozone depletion at the leaf surface after spraying. They added that the residual free water left on the leaf surface after ozone depletion likely promoted proliferation of the pathogen.

Across all our experiments, we implemented nanobubble aeration as the primary method to produce oxygenated and ozonated water treatments. Although direct comparison of aeration methods was not a part of our research goals, existing research highlights nanobubble aeration as an efficient method for generating these treatments. For example, in studying the feasibility of spraying ozonated water treatments for tomato disease prevention, He et al. (2015) found that dissolving ozone in nanobubbles was more effective than using a mixing pump, primarily due to the mechanical shearing of gaseous ozone into nanobubbles that resulted in enhanced dissolution efficiency. Similarly, in comparing aeration methods for wastewater treatment, Xiao and Xu (2020) found a 1.5x increase in oxygen transfer efficiency from nanobubble aeration compared

to coarse bubble aeration methods. Furthermore, Fan et al. (2021) found that gas transfer efficiency of ozonated nanobubbles was 4.7 times higher than that of coarser ozonated bubbles. During our experiments, we found that nanobubble aeration via the NABAS N-5 unit consistently produced high levels of $DO_2$ or $DO_3$ in oxygenated or ozonated water treatments within a matter of 30 minutes, corroborating reported efficiencies of nanobubble technology. Regardless of treatment inefficacies observed in our trials, we maintain that nanobubble aeration is a valuable and viable approach for producing oxygenated or ozonated water.

Conventional methods used to deliver oxygenated or ozonated water treatments in agricultural settings typically include subsurface and aboveground drip irrigation, spray irrigation, drench irrigation, and direct infusion in hydroponic systems via circulating pumps. Among these methods, drip irrigation has likely received the most attention across various cropping systems, especially in studies involving oxygenated water. The underlying principle in implementing this approach is the targeted delivery of oxygen to the root zone to enhance soil oxygen levels, which may lead to more root growth, yield, and potentially less disease (Bhattarai et al., 2005; Zhang et al., 2016). In turfgrass settings, research into the efficacy of using subsurface drip irrigation has been explored since the 1970s (Snyder et al., 1974). However, the technology has never gained widespread market acceptance likely due to problems associated with these systems such as cost of installation, interference with cultivation practices like core aeration, and difficulty in diagnosing problems such as clogs, breaks, or root intrusions (Schiavon et al., 2013; Serena et al., 2014; Sevostianova and Leinauer, 2014). Therefore, we primarily assessed the efficacy of nanobubble water treatments delivered through sprayers. However, we did incorporate soil drench treatments in GCT3 to assess if a different application

method influenced the efficacy of treatments, ultimately finding that the methods yielded similar results.

The lack of efficacy from nanobubble water treatments in our trial could have been due to $DO_2$ loss during spray events. For oxygenated water treatments used in both growth chamber and field trials, we periodically monitored levels of $DO_2$ from the treatment source (i.e. nanobubble-aerated water within sprayer reservoirs) and at the turfgrass surface where spray was directed after passing through a nozzle. During spray events in both field and growth chamber trials, we observed an average $DO_2$ loss of over 65% between water in sprayer reservoirs and water collected at the turf surface. In growth chamber trials utilizing the Generation 3 Research Track Sprayer, the $DO_2$ level in the sprayer reservoir averaged 39.8 mg $L^{-1}$ but fell to an average of 12.7 mg $L^{-1}$ at the turf surface after spraying (68% loss of $DO_2$). In field trials utilizing a handheld $CO_2$-pressurized boom sprayer, the $DO_2$ level in the sprayer reservoir averaged 39.5 mg $L^{-1}$ but fell to an average of 11.1 mg $L^{-1}$ at the turf surface after spraying (72% loss of $DO_2$). Despite these substantial reductions, $DO_2$ levels of oxygenated water treatments after spraying still remained higher than $DO_2$ levels of tap water treatments utilized across all trials (6.5-8.0 mg $L^{-1}$). Recently, in studying the effects of oxygenated nanobubble water irrigation on creeping bentgrass (*Agrostis stolonifera* L.) putting greens, DeBoer et al. (2024) reported similar findings regarding $DO_2$ loss during application with an irrigation hose. They tracked $DO_2$ levels of irrigation water from the nanobubble aeration tank to the turf surface and reported an average $DO_2$ loss of 55% at the turf surface during irrigation events. They also reported that oxygenated nanobubble water did not improve plant health characteristics of creeping bentgrass in the field or greenhouse and did not affect overall soil oxygen content across the majority of their trials. In our trials, the decrease in $DO_2$ levels we observed during spray events may be attributed to off-

gassing, a process where dissolved gases escape from liquid into the atmosphere. Changes in pressure can affect off-gassing by influencing the solubility of gas in a liquid (Markham and Kobe, 1941). The transitioning of aqueous nanobubble treatments through a high-pressure spray nozzle into the lower-pressured atmospheric environment may have led to substantial off-gassing in our trials.

**Conclusion**

This study aimed to examine the effects of oxygenated and ozonated nanobubble water treatments on turf quality and dollar spot suppression in seashore paspalum. While these treatments did not negatively impact turfgrass health, they were ineffective in reducing dollar spot disease severity across all experiments. Despite these results, nanobubble aeration proved to be an efficient method in generating oxygenated and ozonated water treatments for the purposes of our study. The observed lack of treatment effectiveness in our experiments may be attributed to inherent infection processes of dollar spot pathogens as well as gaseous loss during spray application events. However, different application methods (spray or soil drench) implemented in a portion of this study did not affect treatment efficacy. The fate of dissolved gases in aqueous solutions under various application conditions is not well understood and warrants further exploration. Furthermore, improvements in application technology, particularly overhead application technology, to mitigate gaseous loss are likely needed to optimize the performance of oxygenated and ozonated water treatments in turfgrass settings. Without these improvements, turfgrass professionals may not be able to capitalize on the benefits that oxygenated and ozonated water impart to other cropping systems.

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**Table 4.1:** Overall mean turf quality, green coverage, and Dark Green Color Index (DGCI) values of seashore paspalum 'Sea Isle 1' pots under Oxygenated Nanobubble Water (OXN), Ozonated Nanobubble Water (OZN) and Tap Water (TW) treatments across twelve timepoints in Growth Chamber Trial 1.

| Water Treatment[1] | Frequency of Application | Turf Quality[2] | Green Coverage (%)[3] | DGCI[3] |
|---|---|---|---|---|
| TW | every day | 8.9[a] | 96.4[a] | 0.452[a] |
| TW | every other day | 8.9[a] | 96.7[a] | 0.463[a] |
| TW | every 3 days | 8.9[a] | 97.0[a] | 0.454[a] |
| OXN | every day | 8.8[a] | 95.7[a] | 0.462[a] |
| OXN | every other day | 8.9[a] | 96.6[a] | 0.440[a] |
| OXN | every 3 days | 8.9[a] | 97.3[a] | 0.453[a] |
| OZN | every day | 8.9[a] | 96.4[a] | 0.446[a] |
| OZN | every other day | 8.9[a] | 95.8[a] | 0.438[a] |
| OZN | every 3 days | 8.9[a] | 97.2[a] | 0.464[a] |

[1]Water treatments: OXN= Oxygenated Nanobubble Water, OZN= Ozonated Nanobubble Water, TW= Tap Water.
[2]Turf quality was visually scored on a scale of 1 to 9 with 1 being dead turfgrass, 6 being minimally acceptable turfgrass, and 9 being dark green dense turf.
[3]Green coverage and Dark Green Color Index (DGCI) were scored via digital imaging analysis using Field Analyzer.
[2,3]Means followed by the same letter do not significantly differ (p = 0.05, Tukey's HSD).

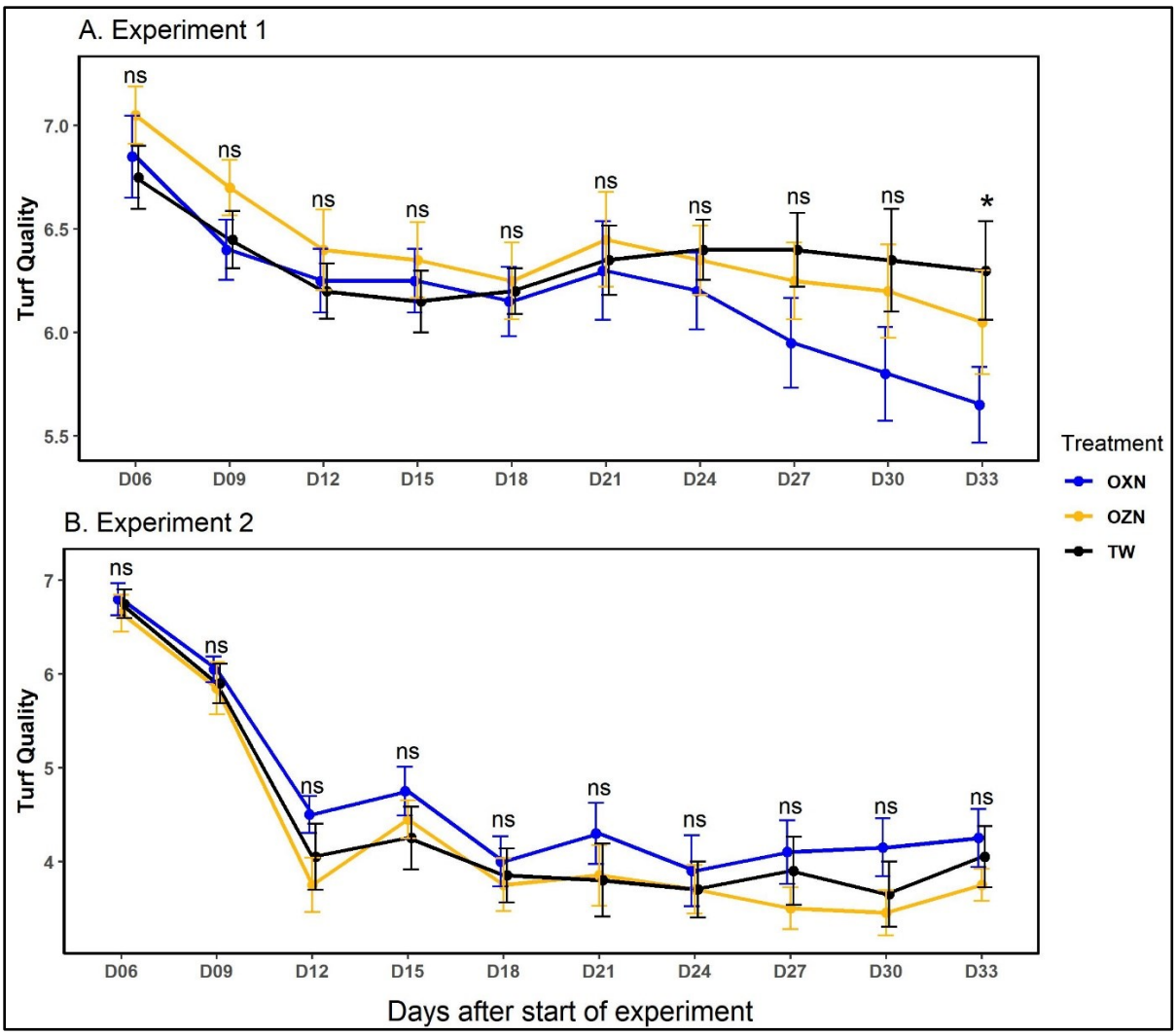


**Figure 4.1:** Mean turf quality progression of dollar spot-inoculated seashore paspalum 'Sea Isle 1' pots under Oxygenated Nanobubble Water (OXN), Ozonated Nanobubble Water (OZN) and Tap Water (TW) treatments, in experiments one (A) and two (B) of Growth Chamber Trial 2. Error bars represent standard error. Statistical significance indicated with '*', non-significance indicated with 'ns' (p = 0.05, Tukey's HSD).

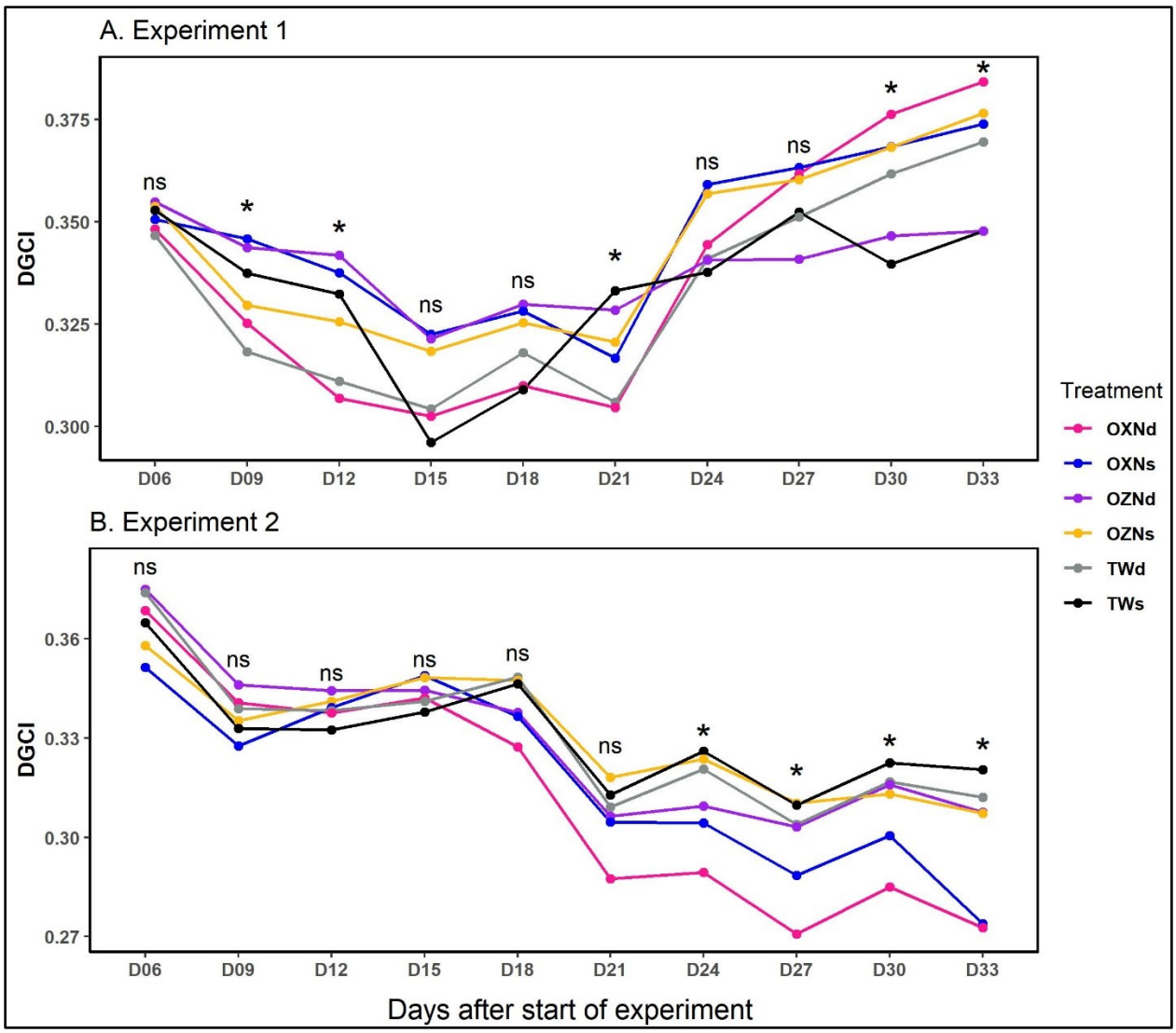


**Figure 4.2:** Mean Dark Green Color Index (DGCI) progression of dollar spot-inoculated seashore paspalum 'Sea Isle 1' pots under three water treatments (Oxygenated Nanobubble Water: OXN, Ozonated Nanobubble Water: OZN, Tap Water: TW) and two application methods (spray: s; drench: d), in experiments one (A) and two (B) of Growth Chamber Trial 3. OXNs= Oxygenated Nanobubble Water Spray, OXNd= Oxygenated Nanobubble Water Drench, OZNs= Ozonated Nanobubble Water Spray, OZNd= Ozonated Nanobubble Water Drench, TWs= Tap Water Spray, TWd= Tap Water Drench. Statistical significance indicated with '*', non-significance indicated with 'ns' (p = 0.05, Tukey's HSD).

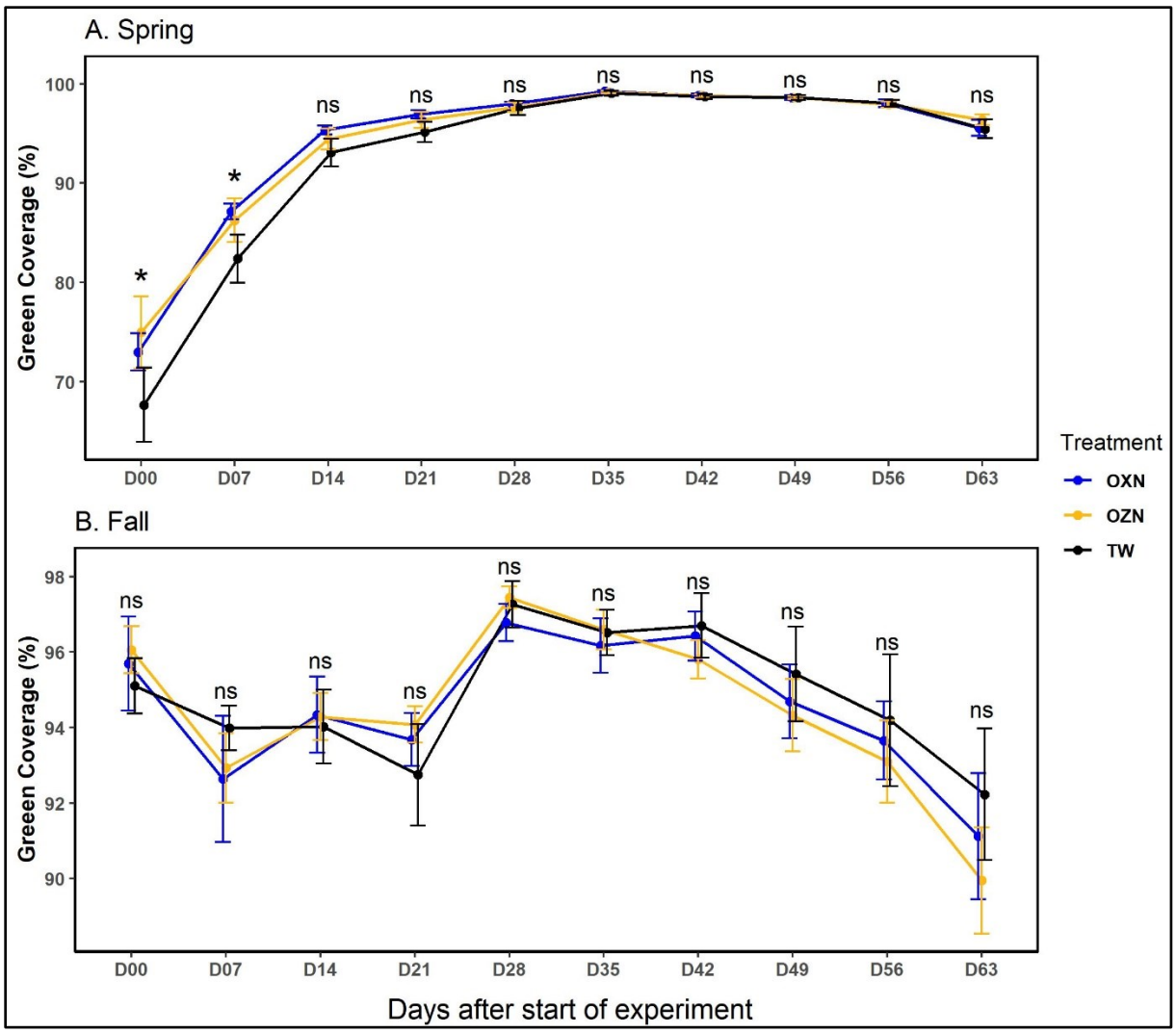


**Figure 4.3:** Mean green coverage progression of seashore paspalum 'Sea Isle 1' plots under Oxygenated Nanobubble Water (OXN), Ozonated Nanobubble Water (OZN) and Tap Water (TW) treatments in spring (A) and fall (B) experiments of Field Trial 1. Error bars represent standard error. Statistical significance indicated with '*', non-significance indicated with 'ns' (p = 0.05, Tukey's HSD).

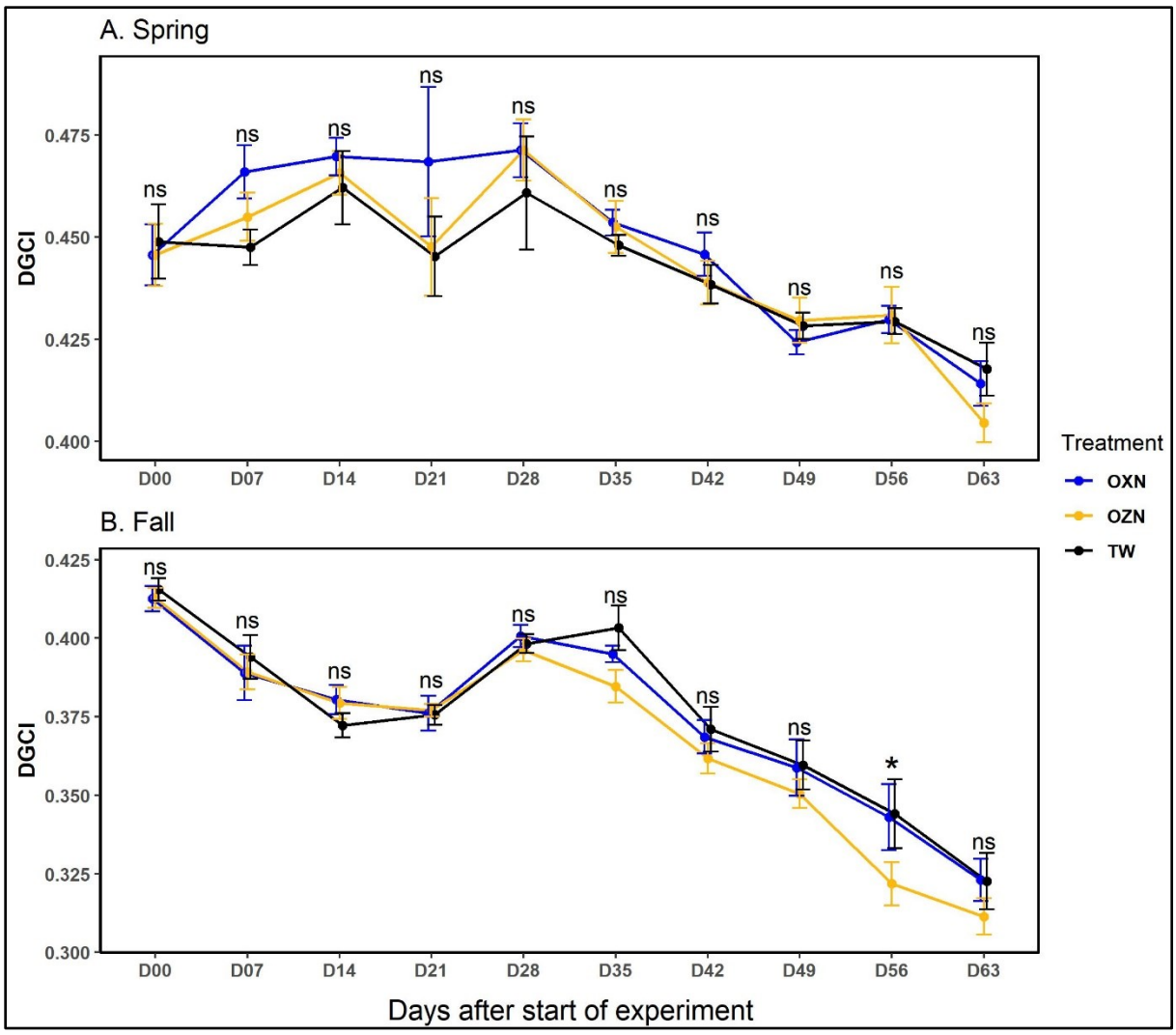


**Figure 4.4:** Mean Dark Green Color Index (DGCI) progression of seashore paspalum 'Sea Isle 1' plots under Oxygenated Nanobubble Water (OXN), Ozonated Nanobubble Water (OZN) and Tap Water (TW) treatments in spring (A) and fall (B) experiments of Field Trial 1. Error bars represent standard error. Statistical significance indicated with '*', non-significance indicated with 'ns' (p = 0.05, Tukey's HSD).

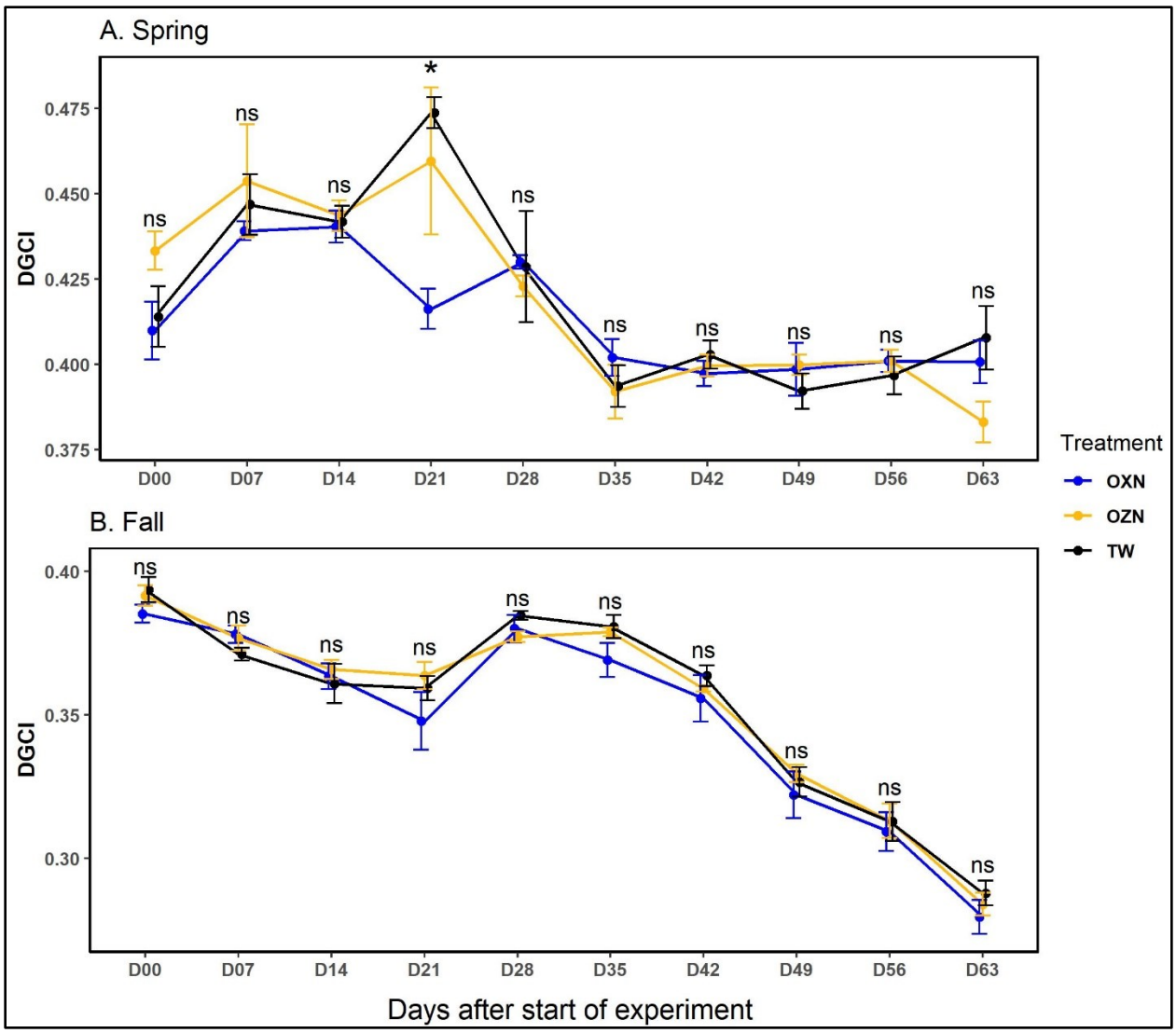


**Figure 4.5:** Mean Dark Green Color Index (DGCI) progression of dollar spot-inoculated seashore paspalum 'Sea Isle 1' plots under Oxygenated Nanobubble Water (OXN), Ozonated Nanobubble Water (OZN) and Tap Water (TW) treatments in spring (A) and fall (B) experiments of Field Trial 2. Error bars represent standard error. Statistical significance indicated with '*', non-significance indicated with 'ns' (p = 0.05, Tukey's HSD).

**Supplemental Materials**

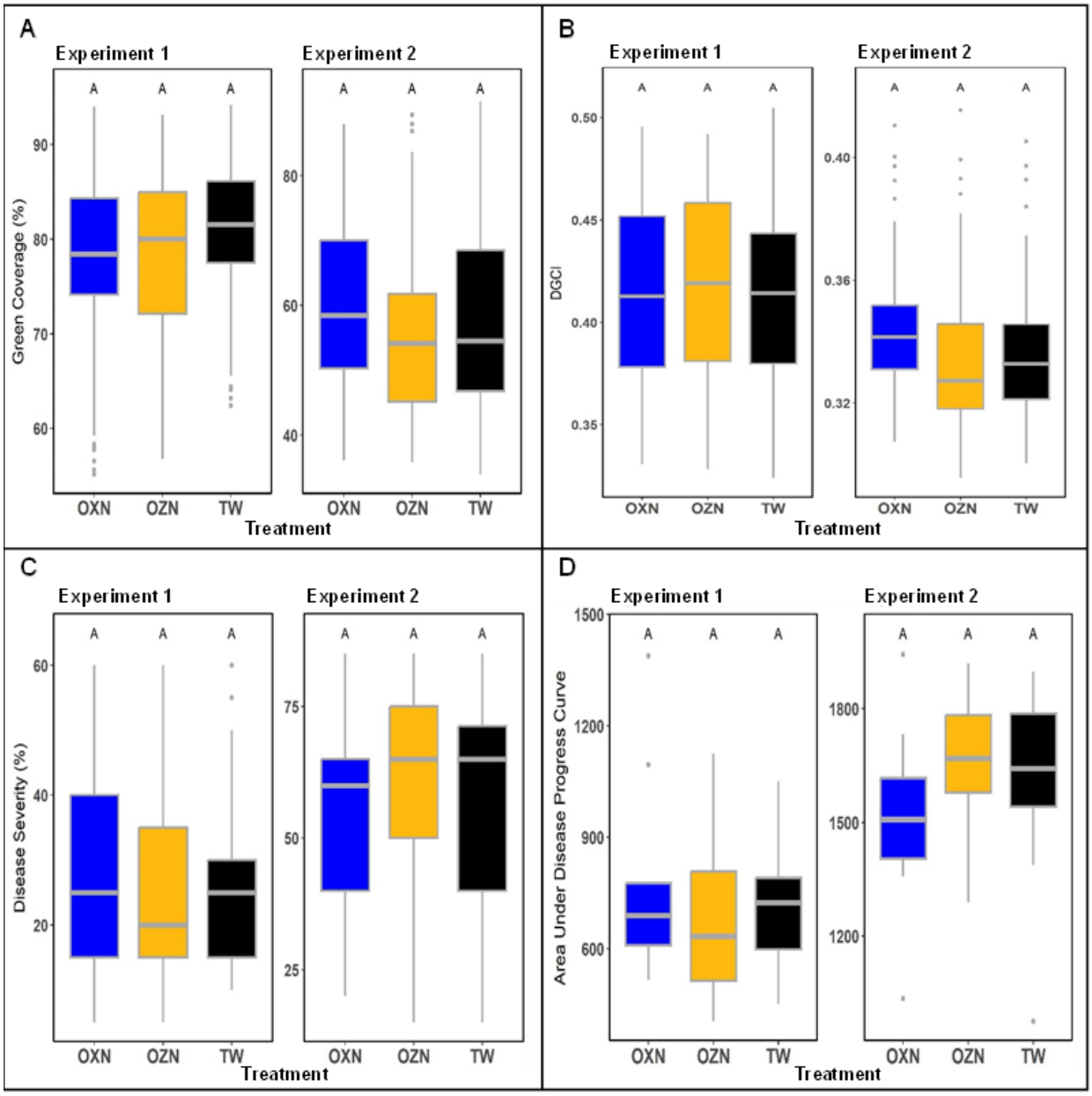


**Figure S4.1:** Mean green coverage (A), Dark Green Color Index (DGCI; B), disease severity (C), and Area Under Disease Progress Curve (AUDPC; D) values of dollar spot-inoculated seashore paspalum 'Sea Isle 1' pots under Oxygenated Nanobubble Water (OXN), Ozonated Nanobubble Water (OZN) and Tap Water (TW) treatments, across ten timepoints in experiments one (left) and two (right) of Growth Chamber Trial 2. Values for minimum, maximum, median, and first-third quartiles are shown here. Treatments with the same letter do not significantly differ (p=0.05, Tukey's HSD).

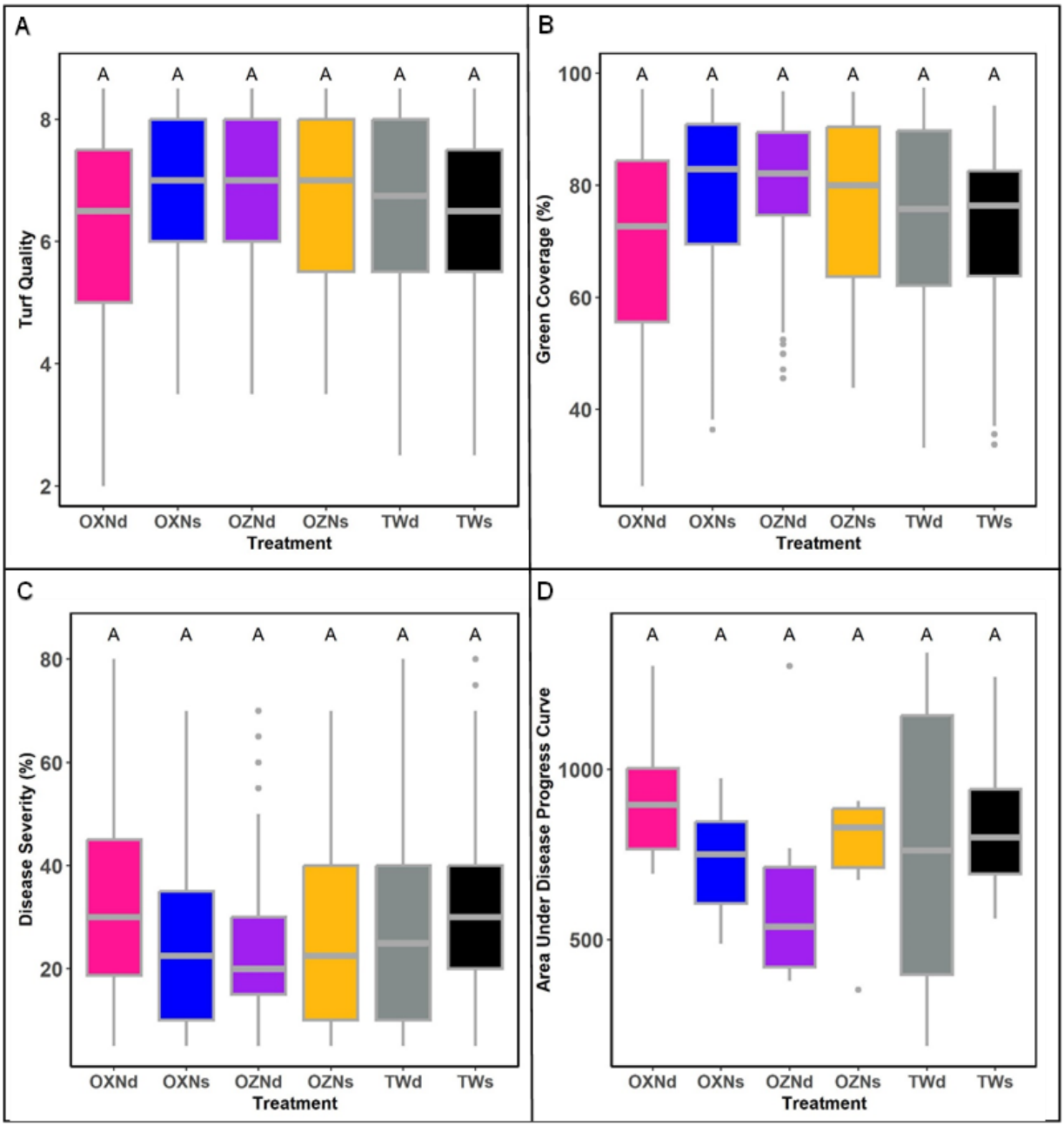


**Figure S4.2:** Mean turf quality (A), green coverage (B), disease severity (C), and Area Under Disease Progress Curve (AUDPC; D) values of dollar spot-inoculated seashore paspalum 'Sea Isle 1' pots under three water treatments (Oxygenated Nanobubble Water: OXN, Ozonated Nanobubble Water: OZN, Tap Water: TW) and two application methods (spray: s; drench: d), across ten timepoints in Growth Chamber Trial 3. Means for each treatment were calculated from combined statistical analysis of duplicated experiments. OXNs= Oxygenated Nanobubble Water Spray, OXNd= Oxygenated Nanobubble Water Drench, OZNs= Ozonated Nanobubble Water Spray, OZNd= Ozonated Nanobubble Water Drench, TWs= Tap Water Spray, TWd= Tap Water Drench. Values for minimum, maximum, median, and first-third quartiles are shown here. Treatments with the same letter do not significantly differ (p=0.05, Tukey's HSD).

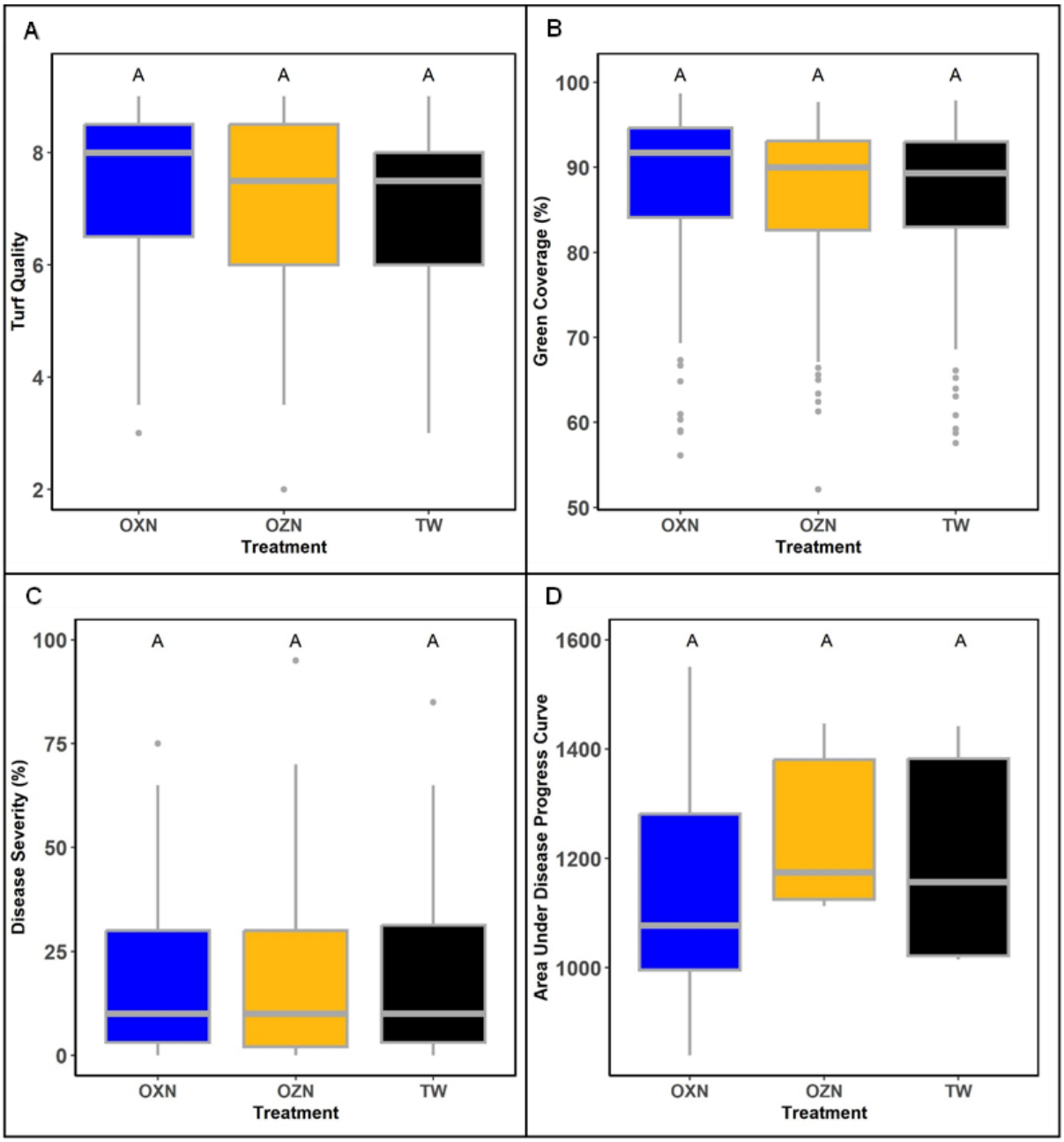


**Figure S4.3:** Mean turf quality (A), green coverage (B), disease severity (C), and Area Under Disease Progress Curve (AUDPC; D) values of dollar spot-inoculated seashore paspalum 'Sea Isle 1' plots under Oxygenated Nanobubble Water (OXN), Ozonated Nanobubble Water (OZN) and Tap Water (TW) treatments across ten timepoints in Field Trial 2. Means for each treatment were calculated from combined statistical analysis of duplicated experiments. Values for minimum, maximum, median, and first-third quartiles are shown here. Treatments with the same letter do not significantly differ (p=0.05, Tukey's HSD).

# CHAPTER 5

# CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Since the first official report of dollar spot in 1927, it has emerged as one of the most troublesome afflictions of turfgrass across the globe. Unlike most other turfgrass diseases, it affects nearly all cultivated cool- and warm-season turfgrass species at almost any given time during a growing season, making it difficult to monitor and control. Even when timely management strategies are implemented, dollar spot pathogens have a keen ability to rapidly overcome them, especially repetitive fungicide applications. In addition to difficulties in managing the disease, the basic biology and systematics of dollar spot pathogens have been shrouded in confusion for numerous decades. For example, the first official description of the pathogen in 1937 cited production of all sorts of fungal structures, such as apothecia, ascospores, conidia, microconidia, and sclerotial structures, but no other studies since then, except for two documenting spore production in the United Kingdom, have corroborated these findings. Similarly, several past studies inconclusively attributed dollar spot pathogens to various taxonomic genera (e.g. *Rhizoctonia*, *Sclerotinia*, *Poculum*, *Rutstroemia*), which led to additional confusion about where they fit within the fungal tree of life. The recent placement of dollar spot fungi into the newly formed *Clarireedia* genus marks a major milestone in resolving some complications associated with these pathogens, but much work remains to be done in uncovering the physiological, pathological, and genetic nuances of the six species currently belonging to the

genus, as well as new species that will inevitably be discovered. Despite the challenges ahead, researchers can now at least build their work from a common foundation.

The prevalence of dollar spot throughout the southeastern U.S. is remarkable, yet it remains a woefully understudied disease in this region. By evaluating the population structure and diversity of *C. monteithiana* in Georgia, our work contributed to the understanding of the dollar spot pathogen in this part of the country. We found that *C. monteithiana* was the primary causal agent of dollar spot on warm-season turfgrasses in the state, but it was capable of infecting cool-season turfgrasses as well. The same pattern was true for the few *C. jacksonii* isolates we collected, as they were recovered from both cool- and warm-season hosts. To expand on these findings pertaining to host specificity, further sampling of dollar spot isolates from cool-season hosts is needed. Along these lines, it may be particularly insightful to acquire several isolates from locations where overseeding occurs (i.e. planting a cool-season turfgrass species into a warm-season turfgrass species). At such locations, both *Clarireedia* species are likely to coexist, so studying isolates collected from these sites may help clarify the proclivity of each pathogen to infect different cool- or warm-season host types. Furthermore, we observed genetic diversity within *C. monteithiana* by identifying two distinct populations in Georgia using a genotyping-by-sequencing approach. Since this work is the first to utilize a next-generation sequencing technique to evaluate population structure and diversity in *Clarireedia*, we anticipate that other researchers may be able to use our results as a baseline to study other dollar spot pathogen populations throughout the Southeast. Monitoring and rigorous assessment of these populations is arguably more important now than ever due to 1) increasingly stringent fungicide regulations that are altering control tactics, 2) changing environmental conditions, and 3) the

recent taxonomic reclassification that may usher in a new era of species-specific dollar spot management.

In our work assessing alternative management approaches for dollar spot, we observed effective disease control from UV-C radiation applications but not from oxygenated or ozonated nanobubble water treatments. We hope that our research on UV-C can serve as a steppingstone for others to utilize and experiment with this technology, not only because we witnessed significant reductions in dollar spot severity across all trials, but also because we believe turfgrass equipment manufacturers could easily integrate this technology into existing robotic mower platforms, similar to how we modified our robotic mower. We are currently unaware of any manufacturers who have done this, but several companies like Echo, Kress, Worx, and Husqvarna already produce autonomous mowers that are widely used and available to consumers. A few of these companies have recently introduced new technology that allows their robotic mowers to operate in patterns, as opposed to the random operation used in our field trials. A patterned system could offer enhanced disease control by providing a more precise delivery of UV-C radiation to a given area. All that said, before any widespread adoption of UV-C in the turfgrass industry, more research is still needed to evaluate the capabilities, limitations, and long-term effects of this technology in other turfgrass pathosystems. Similar to UV-C, nanobubble technology may also be integrated into existing systems used for turfgrass management, particularly irrigation systems. However, given the gaseous losses we observed through spray applications, a different approach besides overhead irrigation may be necessary to effectively deliver ozonated or oxygenated nanobubble water to turfgrass stands. Subsurface drip irrigation could be a potential solution, but there are several drawbacks surrounding the use of these systems in turfgrass settings, some of which include difficulty in diagnosing problems, increased

maintenance costs, and the need to remove or alter existing turfgrass stands to install them. Perhaps the biggest limitation of subsurface drip irrigation in turfgrass is the inability to aerate over areas where these systems are installed, as doing so would damage lines and other infrastructural components. Another possible avenue for incorporating nanobubbles in turfgrass disease management is using them as fungicide carriers. Nanobubbles are highly stable in aqueous solutions, and incorporating fungicide active ingredients into them could enhance the stability of a fungicide. This could lead to improvements in overall efficacy and efficiency of fungicide applications. This approach may not specifically involve the use of oxygenated or ozonated water, but could still prove effective in disease management nonetheless.

In summary, this work provides new insights into the integrated management of dollar spot. UV-C radiation seems to be a promising alternative for dollar spot control and may help reduce fungicide reliance. In contrast, technological refinements are likely needed for ozonated and oxygenated nanobubble irrigation to be of use in turfgrass systems. The diversity of *C. monteithiana* observed in our population genetic research also has important dollar spot management implications, given the fact that pathogen variability significantly affects fungicide efficacy. Along these lines, understanding pathogen evolutionary biology is essential in breeding for host resistance, which is another important control tactic for dollar spot. Overall, this research advances the understanding and management of dollar spot disease through the exploration of pathogen diversity and novel control strategies.