Biology, Epidemiology, and Management of Spring Dead Spot of Bermudagrass

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Plant Pathology

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March 15th, 2022
Blacksburg, Virginia

Keywords: (turfgrass, plant disease, pathology, *Ophiosphaerella herpotricha*, *Ophiosphaerella korrae*)

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Academic Abstract

Spring dead spot (*Ophiosphaerella* spp.) (SDS) of bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) is one of the most challenging diseases in the United States transition zone. Six projects were conducted from 2019 to 2022 to better understand the environmental, edaphic, and spatial distribution of SDS epidemics and to examine management strategies for SDS with chemical and cultural practices. A survey of 51 locations provided support of the geographic distribution of *Ophiosphaerella* species across the Mid-Atlantic United States. *Ophiosphaerella herpotricha* and *O. korrae* were isolated from the Mid-Atlantic region, yet *O. narmari* was not. Cultivars in which parent material originated from the midwestern United States had predominantly *O. herpotricha* and cultivars in which the parent material originated from the southeastern United States had predominantly *O. korrae*. *In vitro* and *in situ* fungicide efficacy screenings were conducted for *O. herpotricha* and *O. korrae*. Additionally, field studies were conducted to optimize fungicide applications and bermudagrass recovery from SDS. Results highlighted that, generally, *O. korrae* was less sensitive to fungicides than *O. herpotricha*; the fungicides isofetamid, mefentrifluconazole, penthiopyrad, and pydiflumetofen were generally the most efficacious against SDS; the different fungicide application methods deployed produced mixed results in their effect on fungicide efficacy against SDS with increased efficacy of tebuconazole against SDS with soil surfactant applications and post-application irrigation in certain scenarios; the optimal timing for fungicide applications for SDS was from 13-18°C with tebuconazole and 13-21°C with isofetamid; and nitrogen applications without cultivation practices in the late spring/early summer optimized bermudagrass recovery from SDS. Lastly, a geospatial survey study was conducted to determine the environmental and edaphic factors that influence SDS epidemics. Results were variable with numerous environmental and edaphic factors influencing SDS depending on the year and location; however, soil pH, soil potassium content, and thatch depth were among the most consistent and influential factors on SDS epidemics. Ultimately, these data improve our recommended strategies for successful SDS management.

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General Audience Abstract

Spring dead spot is a damaging turfgrass disease that causes aesthetically displeasing symptoms and potential safety and playability concerns for pedestrians and athletes traversing turfgrass surfaces. This disease is caused by three fungal species, and the distribution of these species in the Mid-Atlantic US and the management of spring dead spot epidemics are not well understood. Studies were conducted from 2019 to 2022 to determine the geographic distribution of the species that cause spring dead spot in the Mid-Atlantic, best management strategies for spring dead spot with chemical and cultural practices, and factors in the environment and soil that influence spring dead spot epidemics. Results from the geographic distribution study showed that two of the three fungal species that cause spring dead spot were found in the Mid-Atlantic US, which has major implications on management strategies for the disease. The results from the studies focusing on best management strategies for spring dead spot with chemical and cultural practices highlight that the two fungal species found in the Mid-Atlantic US responded differently to fungicides, few fungicides suppressed the disease to an acceptable level, fungicide application methods provided variable suppression of the disease, optimal timing for fungicide applications was in the fall months when soil temperatures were between 13°C and 18°C, and nitrogen fertilization without cultivation optimized bermudagrass recovery from spring dead spot symptoms. Lastly, the study examining the environmental and soil factors that influence spring dead spot epidemics showed that many factors in the soil and environment influenced spring dead spot epidemics with soil pH, soil potassium content, and thatch depth among the most prevalent. These studies provide turfgrass managers and researchers a better understanding of spring dead spot and allow for more informed management decisions for prevention of and recovery from the disease.

Dedication

I dedicate this dissertation, first and foremost, to my Lord and Savior Jesus Christ. He has guided me through life in every way, and I am eternally grateful for all He has done. I would have nothing without His grace. I also dedicate this dissertation to my late cousin Eli and his family. Eli has been an incredible inspiration in my life and to many others. I miss him dearly, but I am so excited to see him again one day.

Acknowledgements

I want to thank my love Anica for her unwavering support and encouragement. She has been a forever-steady motivator and encourager to me, and, for that, I am forever grateful. I also want to thank my brother Will, mom Laura Lea, dad Barry, sister-in-law Laura, and nephew James for their incredible support and encouragement during this journey. They have been there for me since Day 1. My family has helped me every step of the way, and I am so thankful for them all and all that they have done for me. Without them, I would not be where I am today. I want to thank my advisor, Dr. David McCall, for everything he has done for me. He gave me a chance to achieve a dream, provided excellent mentorship throughout my time at Virginia Tech, and has helped catapult me into the next phase of my career. I am very thankful to have gained a lifelong mentor and friend in him, and I hope to follow his example in my career. I also want to thank my other committee members: Dr. Jim Kerns, Dr. Mike Goatley, and Dr. Mizuho Nita. Dr. Kerns has been an awesome mentor and friend to me over the last seven years. I am beyond grateful for his support and the motivation he has given me to become the best scientist and, more importantly, best person I can be. Dr. Goatley has exhibited kindness, leadership, and wisdom during my years of knowing him. I am thankful for the example he has set for me and many others. I will take those lessons with me throughout my career and life, and I am very thankful for the mentorship he has provided to me. Dr. Nita has been an incredible mentor and aid to me during my time at Virginia Tech. He has patiently helped me with numerous projects, and he always has a positive and cheerful attitude. His example has been one I hope to follow. I also want to thank my lab members. Kevin Hensler for helping me grow as both a professional and a person. Kevin has become a lifelong mentor and friend to me, and I am forever thankful for the wisdom, advice, and friendship he has given me. Caleb Henderson for always bringing great ideas and laughter to the lab. I will miss his off-the-wall fun facts, great sense of humor, and creative new ideas. I cannot wait to see just how high he can fly throughout his career. Aaron Tucker for sharing exciting new research ideas, talking Braves baseball, and being wise beyond his years. I look forward to what Aaron's bright future holds. Jordan Booth for mentoring me and helping me every step of the way throughout my PhD. He opened my eyes to a totally new perspective on research and extension that I will carry throughout my career. Travis Roberson for always being excited about new ideas and exhibiting unparalleled work ethic. I also want to thank the many collaborators, both internal and external, that I have worked with throughout my PhD. They have given me insight into new ideas and methods that I plan to use in my career. I also want to thank all of the other graduate students, staff, and faculty that have helped me and supported me during my time at Virginia Tech. It has been a life-changing experience, and the people that make up the turf program at Virginia Tech are second to none.

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Chapter 1: Literature Review

Bermudagrass

Bermudagrass (*Cynodon dactylon*) is a perennial warm-season C_4 turfgrass that is widely used for home lawns, athletic fields, and golf courses in the United States (McCarty and Miller, 2002; Zhou et al., 2013). Bermudagrass is also the most widely used turfgrass species for golf course fairways and tee boxes and athletic fields (Yelverton, 2017). Bermudagrass has good traffic tolerance and the ability to recover rapidly from damage, making it ideal for athletic fields and golf courses (McCarty and Miller, 2002; Yelverton, 2017).

Hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) is used for both sports turf and golf courses (Beard, 2002; Reasor et al., 2016). Certain hybrid bermudagrass cultivars have been bred for cold tolerance allowing the region of adaptation for hybrid bermudagrass to expand from the southern parts of the United States well into the northern transition zone (Gopinath et al., 2021; National Turfgrass Evaluation Program, 2017). Cultivars such as 'Latitude 36', 'Patriot', and 'Tahoma 31' are cold tolerant allowing them to be grown further north than many other hybrid bermudagrass cultivars as many hybrid bermudagrass cultivars are susceptible to winter injury (Gopinath et al., 2021; National Turfgrass Evaluation Program, 2017). Aside from winter injury, hybrid bermudagrass is susceptible to the disease spring dead spot (SDS) caused by *Ophiosphaerella* spp. when grown in areas where winter dormancy is induced (Wadsworth and Young, 1960; Walker et al., 2006).

Spring Dead Spot

Spring dead spot was first described in Oklahoma in the mid-twentieth century (Wadsworth and Young, 1960). Aside from North America, the disease has also been reported on many other continents such as Asia, Australia, Europe, and South America (Canegallo, 2016; Geng et al.,

2021; Gullino et al., 2007; Walker and Smith, 1972). Spring dead spot occurs on bermudagrass and other warm-season turfgrass species (Tisserat et al., 1999; Tredway and Butler, 2007; Wadsworth and Young, 1960). Spring dead spot is a problematic disease to bermudagrass grown in the transition zone of the United States, and symptoms appear as circular, sunken, and necrotic patches on bermudagrass at spring greenup (Tredway et al., 2009; Wadsworth and Young, 1960). When SDS symptoms appear, they can increase for up to three years before eventually subsiding in subsequent years (Pair et al., 1986; Tisserat and Fry, 1997). This disease can cause symptoms to both seeded and vegetative bermudagrass cultivars; it also can reduce playability of bermudagrass, particularly for golf courses (Baird et al., 1998; Martin et al., 2001ac). Moreover, certain factors such as thatch, soil compaction, and soil type can influence SDS (Lucas, 1980b; McAfee, 1979; Martin et al., 2001a; Pair et al., 1986). One of the challenges of SDS is that it is caused by three different fungal pathogen species.

Ophiosphaerella Species

There are three fungal species that cause SDS: *O. herpotricha* (Fr:Fr) J. Walker, *O. korrae* (J. Walker & A.M. Smith) Shoemaker & C.E. Babcock (= *Leptosphaeria korrae* J. Walker & A.M. Smith), and *O. narmari* (J. Walker & A.M. Smith) Wetzel, Hulbert, & Tisserat (= *L. narmari* J. Walker & A.M. Smith) (Crahay et al., 1988; Endo et al., 1985; Smith, 1971; Martin et al., 2001a; Tisserat et al., 1989; Walker and Smith, 1972; Wetzel et al., 1999). Morphological identification of this disease is challenging, so uniplex and multiplex primers have been developed to help identify *Ophiosphaerella* spp. via molecular techniques (Martinez et al., 2019; Tisserat et al., 1994; Wetzel et al., 1999). *Ophiosphaerella herpotricha* is the predominant species that causes SDS in the midwestern United States, yet it has also been reported in the southeastern United States (Cottrill et al., 2016; Perry et al., 2008; Tredway et al., 2008; Wetzel et al., 1999). In contrast, *O.*

korrae is the predominant causal agent of SDS in the southeastern United States, but it has been reported in the Midwest and California (Canegallo, 2016; Endo et al., 1985; Iriarte et al., 2004; Perry et al., 2010; Tredway et al., 2008; Wetzel et al., 1999). *Ophiosphaerella narmari* has been reported in the southeastern United States, the Midwest, and California (Iriarte et al., 2004; Wetzel et al., 1999). Only *O. herpotricha* and *O. korrae* have been isolated from bermudagrass in the Mid-Atlantic United States (Hutchens et al., 2019b).

Both *O. herpotricha* and *O. korrae* grow optimally at 20-25°C *in vitro*, yet they most colonize and infect bermudagrass at 15-17°C and cause the most severe damage at < 21°C (Caasi et al., 2010; Crahay et al., 1988; Flores et al., 2015; Perry et al., 2010; Tisserat et al., 1989; Smith, 1971; Walker and Smith, 1972; Walker et al., 2006). Both species infect primarily the bermudagrass stolons and roots via direct hyphal penetration (Caasi et al., 2010; Flores et al., 2015). These species can be a challenge to manage as many soilborne pathogens that infect the roots are difficult to suppress through chemical and cultural management practices.

Although the two species colonize and infect bermudagrass similarly, *O. herpotricha* isolates are generally more aggressive than *O. korrae* isolates, yet *O. korrae* is less suppressed by most tested fungicides than *O. herpotricha* (Iriarte, 2003; Iriarte et al., 2005a; Iriarte et al., 2005b; Tredway et al., 2020). The differential response of *O. herpotricha* and *O. korrae* to fungicides makes chemical management of SDS a challenge.

Chemical Management of Spring Dead Spot

Historically, suppression of SDS with fungicides has been inconsistent (Tredway et al., 2009). There are newer chemistries, particularly isofetamid, that provide consistent SDS suppression, but cost could be a limiting factor for this fungicide (Roberson et al., 2017). However, Booth et al. (2021) demonstrated that precision penthiopyrad applications for SDS suppression are

as effective as blanket applications. Precision applications could increase the feasibility of applying more expensive fungicides for SDS suppression. Older demethylase inhibiting (DMI) chemistries such as fenarimol and tebuconazole have provided inconsistent suppression of SDS (Earlywine and Miller, 2012; Earlywine and Miller, 2013; Shoemaker and Babcock, 1989; Tredway and Butler, 2004; Walker, 2004; Walker et al. 2001, Wetzel, 2000). Additionally, the newer succinate dehydrogenase inhibiting (SDHI) fungicide, penthiopyrad, has also provided inconsistent suppression of SDS (Earlywine and Miller, 2013; Freund et al., 2019; Galle et al., 2019; Stephens et al., 2020). The reported erratic fungicide efficacy against SDS could be from a number of factors—differential responses of *Ophiosphaerella* species to fungicides, application timing, or application method.

It has been reported that *O. herpotricha* and *O. korrae* respond differently to numerous fungicides (Tredway et al., 2020). Tredway et al. (2020) showed that fenarimol, propiconazole, tebuconazole, and propiconazole + azoxystrobin suppressed SDS caused by *O. herpotricha*, yet these fungicides did not suppress SDS caused by *O. korrae*. These results suggest that *O. korrae* is generally less sensitive to fungicides than *O. herpotricha* in the field, but no broad-scale *in vitro* or field fungicide screening has been conducted to test efficacy against *O. herpotricha* and *O. korrae*.

Fungicide application timing for SDS suppression has also been studied (Butler and Tredway, 2006; Lucas, 1980a; Walker, 2009). Butler and Tredway (2006) found that fenarimol suppressed SDS similarly across application timings ranging from August to November. Lucas (1980a) found that benomyl applications made monthly in the fall (October, November, and December) was the only treatment regime to suppress SDS when compared to spring, summer, and winter benomyl applications. Moreover, Walker (2009) determined that two fall applications

of tebuconazole suppressed SDS more than one spring application of tebuconazole. These studies suggest that fall fungicide applications optimally suppress SDS, yet fungicide efficacy against SDS in the field is still inconsistent. No studies have examined how basing fungicide application timing for SDS on calendar-date x soil temperature influences fungicide efficacy against the disease. This may be critical to reducing variability in SDS suppression with fungicides.

Lastly, the fungicide application method may be a factor in the inconsistent efficacy of fungicides against SDS. Soil surfactants can help move pesticide downward in the soil profile, which could help increase fungicide efficacy against SDS (Gannon et al., 2017; Hutchens et al., 2020). Beck et al. (2012) found that applying a soil surfactant with fenarimol and fenarimol + thiophanate-methyl increased their efficacy in comparison to not including a soil surfactant. However, Earlywine and Miller (2015) did not observe added benefit of tank-mixing a soil surfactant with tebuconazole. Post-application irrigation has also been shown to help move pesticide downward in the soil profile suggesting it could help increase fungicide efficacy against SDS (Gannon et al., 2017; Hutchens et al., 2019b; Stephens et al., 2021). However, Butler and Tredway (2006), Kerns et al. (2017), and Walker (2013) did not observe an added benefit of post-application irrigation to fungicide efficacy against SDS. In contrast, Hutchens et al. (2019) observed that post-application irrigation increased azoxystrobin efficacy against the root disease summer patch (*Magnaporthiopsis poae*) of creeping bentgrass (*Agrostis stolonifera* L.) in a growth chamber study. Generally, the influence of various fungicide application methods has not consistently increased the efficacy of fungicides against SDS.

Cultural Management Practices for Spring Dead Spot

Chemical management of SDS has not produced consistent results, and the same is true for managing SDS with cultural practices. There have been reports of cultivars that are more tolerant

to SDS than others (Baird et al., 1998; Iriarte et al., 2005b; Martin et al., 2001ab; Pair et al., 1986; Tredway et al., 2009). Cold tolerance of bermudagrass cultivars is related to SDS tolerance (Baird et al., 1998; Martin et al., 2001a; Tredway et al., 2009). Moreover, cultivation, fertilization, and other cultural practices have produced variable effects on SDS. The cultivation practice of aerification has been shown to reduce SDS in one study and increase it in a different study (Perry et al., 2010; Tisserat and Fry, 1997). Vertical mowing can also increase SDS or have no effect on SDS suppression (Perry et al., 2010; Tisserat and Fry, 1997). However, aggressive cultivation practices such as aerification + vertical mowing, sod stripping, or fraze mowing can effectively reduce SDS symptoms the following spring (Miller et al., 2017; Tisserat and Fry, 1997).

The influence of various nutrient applications has not yielded consistent results regarding SDS suppression or recovery. In a Perry et al. (2010) study sulfur applications increased SDS compared to the nontreated control. In contrast, Cottrill et al. (2016) showed that sulfur applications reduced SDS. Tredway et al. (2020) found that sulfur had no effect on SDS. Dernoeden et al. (1991) showed that ammonium sulfate could increase bermudagrass recovery from SDS while McCarty (1992) determined that sulfur-coated urea increased SDS the following year. Manganese has also yielded mixed results on its effect on SDS (Miller et al., 2017; Perry et al., 2010; Tredway et al., 2020). Potassium sulfate increased SDS in a McCarty et al. (1992) study, increased bermudagrass recovery from SDS damage in a Dernoeden et al. (1991) study, and had no effect in a Tredway et al. (2020) study. Nitrogen source can also differentially suppress SDS, but differential effects do not always occur (Cottrill et al., 2016; Dernoeden et al., 1991; Miller et al., 2017; Tredway et al., 2020). Calcium nitrate and ammonium sulfate differentially suppress SDS depending on if *O. herpotricha* or *O. korrae* is the predominant pathogen (Tredway et al., 2020).

Lime and gypsum applications have not been shown to affect SDS (Tredway et al., 2020). However, pH can influence SDS (Dernoeden et al., 1991; Tredway et al., 2020). Moreover, soil pH was negatively correlated with SDS symptoms caused by *O. korrae* and positively correlated with SDS symptoms caused by *O. herpotricha* (Tredway et al., 2020). However, in a Cottrill et al. (2016) study, the optimal growth of *O. korrae in vitro* was at a pH of 6 and the optimal growth of *O. herpotricha in vitro* was from a pH range of 5 to 6 suggesting that the lab results from Cottrill et al. (2016) and field results from Tredway et al. (2020) were different. Regardless, evidence suggests that soil pH is likely playing a role in SDS development.

Research Objectives

The evident inconsistent management of SDS with chemical and cultural practices, the differences in *O. herpotricha* and *O. korrae*, and the lack of understanding of SDS epidemics led the authors to address the six research objectives listed below for this dissertation.

1. Survey the geographic distribution of *Ophiosphaerella* species in the Mid-Atlantic United States.
2. Determine the response of *O. herpotricha* and *O. korrae* to fungicides *in vitro* and *in situ*.
3. Examine the influence of soil surfactant and post-application irrigation on tebuconazole efficacy against SDS.
4. Determine the optimal fungicide application timing based on season and soil temperature.
5. Elucidate the environmental and edaphic factors that influence SDS epidemics.
6. Examine the effects of fertility and cultivation on bermudagrass recovery from SDS damage.

References

Baird, J.H., Martin, D.L., Taliaferro, C.M., Payton, M.E., and Tisserat, N.A. 1998. Bermudagrass resistance to spring dead spot caused by *Ophiosphaerella herpotricha*. Plant Dis. 82:771-774. https://doi.org/10.1094/PDIS.1998.82.7.771

Beard, J.B. 2002. Turf Management for Golf Courses, 2nd edition. Chelsea, MI.

Beck, L.L., Moore-Kucera, J., Henry, G., Woodward, J., Zak, J., and Cox, R. 2012. Evaluation of Chemical and Cultural Methods for the Management of Spring Dead Spot in Bermudagrass Turf. Dissertation. Texas Tech Univ., Lubbock, TX. Retrieved from https://ttu-ir.tdl.org/bitstream/handle/2346/50750/Beck_Leslie_Diss.pdf?sequence=1&isAllowed=y

Butler, E.L. and Tredway, L.P. 2006. Method and Timing of Fungicide Applications for Control of Spring Dead Spot in Hybrid Bermudagrass. Online. Plant Health Prog. doi:10.1094/PHP-2006-0901-01-RS.

Caasi, O.C., Walker, N.R., Marek, S.M., Enis, J.N., and Mitchell, T.K. 2010. Infection and colonization of turf-type bermudagrass by *Ophiosphaerella herpotricha* expressing green or red fluorescent proteins. Phytopathology 100:415-423. doi:10.1094/PHYTO-100-5-0415.

Canegallo, A.L. 2016. Characterization and control of *Ophiosphaerella* spp. causing spring dead spot of bermudagrass in South Carolina, USA and Buenos Aires, Argentina. PhD diss. Clemson Univ., Clemson, SC.

Cottrill, D.J., Earlywine, D.T., and Miller, G.L. 2016. Assessment of nitrogen source, sulfur, and fall fungicide applications on the management of spring dead spot of bermudagrass. Plant Dis. 100:473-482. doi:http://dx.doi.org/10.1094/PDIS-05-15-0565-RE

Crahay, J.N., Dernoeden, P.H., and O'Neill, N.R. 1988. Growth and pathogenicity of *Leptosphaeria korrae* in bermudagrass. Plant Dis. 72:945-949.

Dernoeden, P.H., J.N. Crahay, and D.B. Davis. 1991. Spring dead spot and bermudagrass quality as influenced by nitrogen source and potassium. Crop Sci. 31:1674-1680. https://doi.org/10.2135/cropsci1991.0011183X003100060058x

Earlywine, D.T. and Miller, G.L. 2012. Evaluation of fungicides for spring dead spot control on bermudagrass, 2010-2011. Plant Dis. Manag. Rep. 6:T022.

Earlywine, D.T. and Miller, G.L. 2013. Evaluation of fungicides for spring dead spot control on bermudagrass, 2011-2012. Plant Dis. Manag. Rep. 7:T008.

Earlywine, D.T. and Miller, G.L. 2015. Evaluation of multiple fungicides in combination with a wetting agent for spring dead spot control on bermudagrass, 2013-2014. Plant Dis. Manag. Rep. 9:T018.

Endo, R.M., Ohr, H.D., and Krausman, E.M. 1985. *Leptosphaeria korrae*, a cause of the spring dead spot disease of bermudagrass in California. Plant Dis. 69:235-237.

Flores, F.J., Marek, S.M., Anderson, J.A., Mitchell, T.K., and Walker, N.R. 2015. Infection and colonization of several bermudagrasses by *Ophiosphaerella korrae*. Phytopathology 105:656-661. http://dx.doi.org/10.1094/PHYTO-07-14-0205-R

Freund, D.R., Kerns, J.P., Butler, E.L., and Ploetz, J.N. 2019. Evaluation of fungicides for control of spring dead spot on a bermudagrass putting green, 2017-2018. Plant Dis. Manag. Rep. 13:T005.

Galle, G.H., Kerns, J.P., Butler, E.L., and Ploetz, J.N. 2019. Evaluation of Kabuto and Tekken for the control of spring dead spot on ultradwarf bermudagrass putting greens, 2017-2018. Plant Dis. Manag. Rep. 13:T006.

Gannon, T.W., Jeffries, M.D., and Ahmed, K.A. 2017. Irrigation and Soil Surfactants Affect Abamectin Distribution in Soil. Crop Sci. 57.2:573-580. https://doi.org/10.2135/cropsci2016.05.0320

Geng, J.M., Jiang, S., and Hu, J. 2021. First Report of *Ophiosphaerella narmari* Causing Spring Dead Spot of Hybrid Bermudagrass in China. Plant Dis. 105:4153. https://doi.org/10.1094/PDIS-03-21-0535-PDN

Gullino, M.L., Mocioni, M., and Titone, P. 2007. First Report of *Ophiosphaerella korrae* Causing Spring Dead Spot of Bermudagrass in Italy. Plant Dis. 91:1200. https://doi.org/10.1094/PDIS-91-9-1200C

Gopinath, L., Moss, J.Q., and Wu, Y. 2021. Evaluating the freeze tolerance of bermudagrass genotypes. Agrosyst. Geosci. Environ. 4: e20170. https://doi.org/10.1002/agg2.20170

Hutchens, W.J., Gannon, T.W., Shew, H.D., and Kerns, J.P. 2019a. Effect of post-application irrigation on fungicide movement and efficacy against *Magnaporthiopsis poae*. Crop Prot. 122:106-111. https://doi.org/10.1016/j.cropro.2019.04.027

Hutchens, W.J., Gannon, T.W., Shew, H.D., Ahmed, K.A., and Kerns, J.P. 2020. Soil surfactants influence fungicide movement in United States Golf Association putting green soil. J. Environ. Qual. 49:450-459. https://doi.org/10.1002/jeq2.20021

Hutchens, W.J., Henderson, C.A., Bush, E.A., and McCall, D.S. 2019b. Geographic Distribution of *Ophiosphaerella* Species in the Mid-Atlantic. Abstract. *Crop Science Society of America* Annual Meeting, 2019.

Iriarte, F.B. 2003. Genetic diversity and aggressiveness of *Ophiosphaerella korrae*, a cause of spring dead spot of bermudagrass. PhD diss. Kan. State Univ., Manhattan, KS.

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., and Tisserat, N.A. 2004. Genetic diversity and aggressiveness of *Ophiosphaerella korrae*, a cause of spring dead spot of bermudagrass. Plant Dis. 88:1341-1346.

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., Vincelli, P., Dixon, E.W., and Tisserat, N.A. 2005a. Aggressiveness of spring dead spot pathogens to bermudagrass. Int. Turf. Res. J. 10:258-264.

Iriarte, F.B., Fry, J.D., Martin, D.L., Todd, T.C., and Tisserat, N.A. 2005b. Effect of cold acclimation and freezing on spring dead spot severity in bermudagrass. HortScience 40:421-423.

Lucas, L.T. 1980a. Control of spring dead spot of bermudagrass with fungicides in North Carolina. Plant Dis. 64:868-870.

Lucas, L.T. 1980b. Spring dead spot of bermudagrass. p. 183-187. *In* P.O. Larsen and B.G. Joyner (ed.) Advances in turfgrass pathology. Harcourt Brace Jovanovich, Duluth, MN.

Martin, D.L., Bell, G.E., Baird, J.H., Taliaferro, C.M., Tisserat, N.A., Kuzmic, R.M., Dobson, D.D., and Anderson, J.A. 2001a. Spring Dead Spot Resistance and Quality of Seeded Bermudagrasses under Different Mowing Heights. Crop Sci. 41:451-456.

Martin, D.L., Bell, G.E., Taliaferro, C.M., Tisserat, N.A., Baird, J.H., Dobson, D.D., Kuzmic, R.M., and Anderson, J.A. 2001b. Spring dead spot resistance of inter-specific hybrid bermudagrasses J. Intl. Turf. Res. 9:3-6.

Martin, D.L., Taliaferro, C.M., Tisserat, N.A., Bell, G.E., Baird, J.H., Dobson, D.D., Anderson, J.A., and Kuzmic, R.M. 2001c. Hardy bermudagrasses sought with resistance to spring dead spot. Golf Course Management: June 2001 edition.

Martinez, J.F.I., Flores, F.J., Koch, A.R., Garzon, C.D., and Walker, N.R. 2019. Multiplex end-point PCR for the detection of three species of *Ophiosphaerella* causing spring dead spot of bermudagrass. Plant Dis. 103:2010-2014. https.//doi.org/10.1094/PDIS-10-18-1727-RE

McAfee, J. 1979. Proceedings of the Thirty-Fourth Annual Texas Turfgrass Conference p. 23-25.

McCarty, L.B., L.T. Lucas, and J.M. DiPaola. 1992. Spring dead spot occurrence in bermudagrass following fungicide and nutrient applications. HortSci. 27.10:1092-1093. https://doi.org/10.21273/HORTSCI/27.10.1092

McCarty, L.B., and Miller, G.L. 2002. Managing Bermudagrass Turf: Selection, Construction, Cultural Practices, and Pest Management Strategies. Sleeping Bear Press, Chelsea, MI.

Miller, G.L., D.T. Earlywine, and B.S. Fresenburg. 2017. Effect of fraze mowing on spring dead spot caused by *Ophiosphaerella herpotricha* of bermudagrass. Int. Turfgrass Soc. Res. J. 13:225-228. https://doi.org/10.2134/itsrj2016.10.0839

National Turfgrass Evaluation Program. 2017. 2013 National Bermudagrass Test 2013-2017 Data. Final Report NTEP No. 18-14.

Pair, J.C., Crowe, F.J., and Willis, W.G. 1986. Transmission of spring dead spot disease of bermudagrass by turf/soil cores. Plant Dis. 70:877-878.

Perry, D.H., Tomaso-Peterson, M., and Baird, R. 2008. First Report of *Ophiosphaerella herpotricha* Causing Spring Dead Spot of Bermudagrass in Mississippi. Plant Dis. 92:482-483. https://doi.org/10.1094/PDIS-92-3-0482A

Perry, D.H., Tomaso-Peterson, M., and Baird, R. 2010. Seasonal variation in frequency of isolation of *Ophiosphaerella korrae* from bermudagrass roots in Mississippi and pathogenicity and optimal growth of selected isolates. Mycologia 169:395-402. doi:10.1007/s11046-010-9273-x

Reasor, E.H., Brosnan, J.T., Trigiano, R.N., Elsner, J.E., Henry, G.M., and Schwartz, B.M. 2016. The genetic and phenotypic variability of interspecific hybrid bermudagrasses (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) used on golf course putting greens. Planta 244:761-773. https://doi.org/10.1007/s00425-016-2573-8

Roberson, T.L., McCall, D.S., Estes, A., and Shelton, C.D. 2017. Novel Spring Dead Spot Control Using Isofetamid. Abstract. *Crop Science Society of America* Annual Meeting, 2017.

Shoemaker, R.A., and Babcock, C.E. 1989. *Phaeosphaeria*. Can. J. Bot. 67:1500-1599.

Smith, A.M. 1971. Control of spring dead spot of couch grass turf in New South Wales. J. Sports Turf Res. Inst. 47:60-65.

Stephens, C.M., Kerns, J.P., Ahmed, K.A., and Gannon, T.W. 2021. Influence of post-application irrigation and mowing timing on fungicide fate on a United States Golf Association golf course putting green. J. Environ. Qual. 50:868-876. https://doi.org/10.1002/jeq2.20249

Stephens, C.M., Ploetz, J.N., Butler, E.L., and Kerns, J.P. 2020. Evaluation of fungicides for the control of spring dead spot on ultradwarf bermudagrass putting greens, 2018-2019. Plant Dis. Manag. Rep. 14:T011.

Tisserat, N.A. and J.D. Fry. 1997. Cultural practices to reduce spring dead spot (*Ophiosphaerella herpotricha*) severity in *Cynodon dactylon*. J. Intl. Turf. Res. 8:931-936.

Tisserat, N.A., Hulbert, S.H., and Sauer, K.M. 1994. Selective Amplification of rDNA Internal Transcribed Spacer Regions to Detect *Ophiosphaerella korrae* and *O. herpotricha*. Phytopathology 84:478-482.

Tisserat, N.A., Pair, J.C., and Nus, A. 1989. *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermudagrass in Kansas. Plant Dis. 73:933-937.

Tisserat, N.A., Wetzel H. III., Fry, J., and Martin D.L. Spring Dead Spot of Buffalograss Caused by *Ophiosphaerella herpotricha* in Oklahoma. Plant Dis. 83:199. https://doi.org/10.1094/PDIS.1999.83.2.199D

Tredway, L.P., and Butler, E.L. 2007. First Report of Spring Dead Spot of Zoysiagrass Caused by *Ophiosphaerella korrae* in the United States. Plant Dis. 91:1684. https://doi.org/10.1094/PDIS-91-12-1684A

Tredway, L.P., Butler, E.L., Soika, M.D., and Bunting, M.L. 2008. Etiology and management of spring dead spot of hybrid bermudagrass in North Carolina, USA. Acta Hort. 783:535-546.

Tredway, L.P., Soika, M.D., Butler, E.L., and Kerns, J.P. 2020. Impact of nitrogen source, fall fertilizers, and preventative fungicides on spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha*. Crop Sci. Special Issue: International Turfgrass Research Conference: 1-10. doi:10.1002/csc2.20306

Tredway, L. P., Tomaso-Peterson, M., Perry, H., and Walker, N. R. 2009. Spring dead spot of bermudagrass: A challenge for researchers and turfgrass managers. Online. Plant Health Prog. doi:10.1094/PHP-2009-0710-01-RV.

Wadsworth, D. F. and Young, H. C. 1960. Spring dead spot of bermudagrass. Plant Dis. 44:516-518.

Walker, J., and Smith, A.M. 1972. *Leptosphaeria narmari* and *L. korrae* spp. nov., Two Long-Spored Pathogens of Grasses in Australia. Trans. Br. Mycol. Soc. 58:459-466.

Walker, N.R. 2004. Evaluation of fungicides for the management of spring dead spot of bermudagrass, 2003-2004. Fung. Nemat. Tests 60:T022.

Walker, N.R. 2009. Influence of fungicide application timings on the management of bermudagrass spring dead spot caused by *Ophiosphaerella herpotricha*. Plant Dis. 93:1341-1345.

Walker, N.R., Jackson, K.E., and Martin, D.L. 2001. Evaluation of fungicides for the management of spring dead spot of common bermudagrass turf, 2000-2001. Fung. Nemat. Tests 57:T12.

Walker, N.R., Mitchell, T.K., Morton, A.N., and Marek, S.M. 2006. Influence of temperature and time of year on colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. Plant Dis. 90:1326-1330.

Wetzel, H.C. 2000. Evaluation of fungicides for control of spring dead spot of bermudagrass, 2000. Fung. Nemat. Tests 56:T3.

Wetzel, H.C., Hulbert, S.H., and Tisserat, N.A. 1999. Molecular evidence for the presence of *Ophiosphaerella narmari* n. comb., a cause of spring dead spot of Bermudagrass, in North America. Mycol. Res. 103:981-989. https://doi.org/10.1017/S0953756298007977

Wetzel, H.C., III, Skinner, D.Z., and Tisserat, N.A. 1999. Geographic distribution and genetic diversity of three *Ophiosphaerella* species that cause spring dead spot of bermudagrass. Plant Dis. 83:1160-1166.

Yelverton, F. 2017. Bermudagrass. NC State Extension Publications. https://content.ces.ncsu.edu/bermudagrass.

Zhou, Y., Lambrides, C.J., and Fukai, S. 2013. Drought Resistance of C_4 Grasses Under Field Conditions: Genetic Variation Among a Large Number of Bermudagrass (*Cynodon* spp.) Ecotypes Collected from Different Climatic Zones. J. Agro. Crop. Sci. 199:253-263. https://doi.org/10.1111/jac.12020

Chapter 2: Geographic Distribution of *Ophiosphaerella* Species in the Mid-Atlantic United States

Abstract

Spring dead spot (SDS) of bermudagrass (*Cynodon dactylon*) is primarily caused by *Ophiosphaerella herpotricha* and *Ophiosphaerella korrae* in North America. These two species respond differently to numerous management practices, grow optimally at different soil pH ranges, and differ in aggressiveness. Understanding the *Ophiosphaerella* species distribution in regions where SDS occurs will allow turfgrass managers to tailor their management practices toward the predominant species present. A survey was conducted in the Mid-Atlantic United States in which one to 14 samples of bermudagrass expressing SDS symptoms were taken from 51 athletic fields, golf courses, or sod farms across Delaware, Maryland, North Carolina, and Virginia. DNA was isolated from necrotic root and stolon tissue, amplified using species-specific primers, and detected in a real-time PCR assay. At least one isolate of *O. herpotricha* was recovered from 76% of the locations and *O. korrae* was recovered from 73% of the locations. *Ophiosphaerella herpotricha* was amplified from 55% of the samples while *O. korrae* was amplified from 37% of the samples. There were distinct regions in the Mid-Atlantic in which either *O. herpotricha* or *O. korrae* was predominant. *Ophiosphaerella herpotricha* was predominant in western Virginia, central North Carolina as well as Delaware and eastern Maryland. However, *O. korrae* was predominant in central Maryland and Virginia as well as eastern Virginia and North Carolina. *Ophiosphaerella herpotricha* was isolated from certain cultivars more frequently than *O. korrae* and vice versa. These survey results elucidate the geographic distribution of *O. herpotricha* and *O. korrae* throughout the Mid-Atlantic United States.

Introduction

The turfgrass disease spring dead spot (SDS) is caused by *Ophiosphaerella* spp. and is detrimental to bermudagrass (*Cynodon* spp.) in regions where winter induced dormancy of the grass occurs (Tisserat et al., 1989). *Ophiosphaerella* spp. infect primarily during the fall and damage appears as bermudagrass transitions out of dormancy in the spring (Walker et al., 2006). Typical symptoms are circular, straw-colored patches ranging from a few centimeters to a meter or more in diameter (Dernoeden et al., 1991). These patches are often sunken, which may cause playability and safety concerns for golfers, athletes, and pedestrians navigating symptomatic turfgrass stands. Aside from the functional problems that SDS can cause, it also reduces aesthetic value of turfgrass, which further underscores the necessity to understand and manage this disease.

Reports from turfgrass professionals throughout the United States via an online survey suggest that SDS occurs farther north than previously known (personal communication) (Fig. 1). In the online survey turfgrass professionals reporting SDS in New York and Wisconsin were anomalies, which could have been from misidentification of the disease (personal communication). However, there was a report of SDS on a 'Latitude 36' hybrid bermudagrass driving range tee in Pennsylvania suggesting that SDS can occur in more northern regions of the US than previously known (personal communication). Cold tolerant bermudagrass cultivars have extended the area of adaptation farther north for bermudagrass, thereby expanding the distribution of SDS farther north and making it a problematic disease in the Mid-Atlantic region of the United States (Dunne et al., 2019).

There are three *Ophiosphaerella* species that cause SDS: *O. herpotricha* (Fr:Fr) J. Walker, *O. korrae* (J. Walker & A.M. Smith) Shoemaker & C.E. Babcock (=*Leptosphaeria korrae* J. Walker & A.M. Smith), and *O. narmari* (J. Walker & A.M. Smith) Wetzel, Hulbert, & Tisserat (=*L. narmari* J. Walker & A.M. Smith) (Iriarte et al., 2004; Iriarte et al., 2005a; Wetzel et al.,

1999). The two most common species in North America are *O. herpotricha* and *O. korrae* with *O. narmari* being the predominant species in Australia and New Zealand (Smith, 1971; Walker and Smith, 1972). Within the United States, species distribution varies by region. Wetzel et al. (1999) documented that *O. herpotricha* was the predominant species in Oklahoma and Kansas. Similarly, Cottrill et al. (2016) determined from a sampling survey of Arkansas, Kansas, and Missouri that 93% of the isolates collected (n=154) were *O. herpotricha*. Iriarte et al. (2004) reported *O. korrae* as the most frequently isolated species in the southeastern United States. Similarly, Tredway et al. (2008) determined that *O. korrae* was the most prevalent species isolated from 15 of 19 locations in North Carolina with Canegallo et al. (2016) confirming *O. korrae* as the main causal agent of SDS in South Carolina.

Ophiosphaerella herpotricha is generally more aggressive than *O. korrae* in growth chamber, greenhouse, and field studies (Iriarte, 2003; Iriarte et al., 2005a; Iriarte et al., 2005b). It was found that *O. herpotricha* isolates generally caused larger necrotic patch diameters in the field than *O. korrae* isolates (Iriarte et al., 2005a). Furthermore, it was determined that *O. herpotricha* caused greater disease severity than *O. korrae* in a growth chamber study (Iriarte et al., 2005b).

The two species also differ in their response to various management practices. Tredway et al. (2020) showed that calcium nitrate suppressed *O. korrae*, while ammonium sulfate suppressed *O. herpotricha* in field plots that were inoculated. This may have been caused by the effect that the nitrogen sources had on the soil pH, since soil pH affects *Ophiosphaerella* species differently (Cottrill et al., 2016). Moreover, the two species respond differently to numerous fungicides *in vitro* and *in vivo* with fungicides from the demethylase inhibitors (DMIs), quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs) differentially suppressing *O. herpotricha* and *O. korrae* (Hutchens et al., 2019; Hutchens et al., 2020; Tredway et al., 2020).

Although *O. herpotricha* and *O. korrae* vary in the severity of symptoms they cause and their response to certain management practices, optimal temperature for growth and disease development is similar between the two species. Optimal temperature for growth of both *O. korrae* and *O. herpotricha in vitro* is between 20 and 25°C (Crahay et al., 1988; Perry et al., 2010; Tisserat et al., 1989; Walker and Smith, 1972). In contrast to *in vitro* growth, optimal temperature for turfgrass damage caused by the two species is reported to be < 21°C (Crahay et al., 1988; Smith, A.M., 1971; Walker et al., 2006). This may be due to the pathogen outcompeting the bermudagrass plant, which does not grow optimally at temperatures < 21°C, leading to greater disease development (Younger et al., 1972).

The similarities and differences between *O. herpotricha* and *O. korrae* drive turfgrass management decisions and emphasize the importance of understanding their geographic distribution. The southeastern and mid-western United States have been surveyed for the distribution of *O. herpotricha* and *O. korrae*; however, a distribution survey in the Mid-Atlantic United States has never been conducted. The purpose of this study was to determine the geographic distribution of *O. herpotricha* and *O. korrae* in mainly Virginia. as well as the surrounding Mid-Atlantic states.

Materials and Methods

One to 14 hybrid bermudagrass or zoysiagrass (*Zoysia japonica* Steud.) samples were collected from the edge of separate symptomatic patches from 51 turfgrass facilities (e.g., golf courses, athletic fields, sod farms, etc.) throughout Delaware (n = 10), Maryland (n = 60), North Carolina (n = 12), and Virginia (n = 238). A total of 37 locations were sampled in 2013 and 12 locations were sampled in 2019. Two locations, Midlothian, VA and Queenstown, MD, were sampled in both 2013 and 2019, and data from both years were grouped together. Samples were

immediately placed into a cooler until they could be transferred into a -20°C freezer, where they were stored until DNA extraction was initiated. Necrotic stolons and roots collected from each sample were homogenized after freezing in liquid nitrogen using a mortar and pestle. DNA was isolated using Qiagen DNeasy plant mini-kit [Qiagen, Hilden, Germany] and DNA quality and quantity were measured using a Nanodrop [Thermo Fisher Scientific, Waltham, MA].

After DNA extraction and isolation, a qPCR assay was conducted. For the samples collected in 2013, qPCR reactions were conducted using only the OHITS and OKITS primers [Thermo Fisher Scientific, Waltham, MA] developed by Tisserat et al. (1994) (Table 1). For the 2019 samples, qPCR reactions were conducted using either the OHITS and OKITS primers or the OHER and OKOR primers [Thermo Fisher Scientific, Waltham, MA] developed by Martinez et al. (2019) (Table 1). The OHER and OKOR primers were used for the 2019 samples because they were newly developed and designed to amplify different regions of the genome for *O. herpotricha*, *O. korrae,* and *O. narmari* thereby reducing the likelihood of non-specific binding. Sample DNA was added to 20 µl singleplex reactions with primers designed to separate *O. herpotrica* and *O. korrae* (Martinez et al., 2019; Tisserat, 1994). Reactions consisted of ~30-40 ng of template DNA, 1µM of primers, 10µl of SsoAdvanced Universal SYBR Green Supermix [Bio-Rad Laboratories, Hercules, California, USA], and water to make a 20µl reaction. The qPCR assays were performed using the API-7300 [Applied Biosystems, Foster City, California, USA] with an initial 2-minute cycle of 98°C followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Melt-curve analysis was used to confirm PCR amplification products as target. Locations from the 2019 survey where all samples failed to amplify with the OHITS and OKITS primers were also assayed using the OHER and OKER primers, as well as the *O. narmari* (ONAR) primers developed by Martinez et al. (2019). Samples that failed to amplify under either of the assays outlined above were not

used in further analysis. Detection frequency of each species was based on the number of samples that amplified for each species per total number of samples processed. All samples were processed either at the Virginia Tech Glade Road Research Facility, Blacksburg, VA or the Virginia Tech Plant Disease Clinic, Blacksburg, VA.

Coupling the isolation frequency data with GPS coordinates from the locations sampled, a heat map was created using the universal cokriging analysis method with *O. herpotricha* isolation frequency as the main variable of interest and *O. korrae* isolation frequency as the covariate in ArcGIS software [Esri, Redlands, California, USA] (Martinez-Murillo et al., 2017). An exponential model was used for spatial dependence and eight classes were created using the geometric interval classification method. Additionally, isolation frequency of each *Ophiosphaerella* species for each hybrid bermudagrass cultivar was assessed with analysis of variance (ANOVA). Only hybrid bermudagrass cultivars with at least three different locations sampled for *Ophiosphaerella* species frequency were analyzed with each location representing a replication. Means were separated using the Student's *t*-test in JMP Pro 15 [SAS Institute, Cary, NC, USA].

Results

Of the 51 locations sampled, DNA sequences from either *O. herpotricha* or *O. korrae* were amplified in at least one sample. *Ophiosphaerella herpotricha* DNA sequence was amplified from 76% of the locations, and *O. korrae* DNA sequence was amplified from 73% of the locations (Table 2). Both species' DNA sequences were amplified from 49% of the locations, and the DNA sequence for *O. herpotricha* alone was amplified from 27% of the locations, while the DNA sequence for *O. korrae* alone was amplified from 24% of the locations.

In Delaware only two locations were sampled. Across both locations the DNA sequence for *O. herpotricha* was amplified in 80% of the samples and the DNA sequence for *O. korrae* was amplified in 30% of the samples. A total of nine locations were sampled across Maryland from which the DNA sequence for *O. herpotricha* was amplified in 63% of the samples and the DNA sequence for *O. korrae* was amplified in 15% of the samples. Three locations were sampled in North Carolina, and the DNA sequence for *O. herpotricha* was amplified in 25% of the samples while the DNA sequence for *O. korrae* was amplified in 75% of the samples. There were 37 locations sampled across Virginia with the DNA sequence for *O. herpotricha* and *O. korrae* amplified in 53% and 40% of the samples, respectively.

The predominance of either *O. herpotricha* or *O. korrae* was clustered in distinct regions throughout the Mid-Atlantic based on cokriging analysis of isolation frequency of each species (Fig. 2). Delaware, the western region of Virginia, central North Carolina, and the eastern peninsula of Maryland had a high proportion of *O. herpotricha*. In contrast, western Maryland and central and southeastern Virginia and North Carolina had predominantly *O. korrae* populations.

The cultivars of bermudagrass or zoysiagrass were determined for 46 of the 51 locations sampled, with samples from the remaining five locations coming from unknown cultivars. There were 10 different bermudagrass cultivars identified: ‘Celebration,’ ‘Common,’ ‘Latitude 36,’ ‘Norman,’ ‘Patriot,’ ‘Riviera,’ ‘TifEagle,’ ‘TifSport,’ ‘Tifway 419,’ and ‘Vamont.’ Only one location had zoysiagrass, and the cultivar was ‘Empire.’ The isolation frequencies of *O. herpotricha* and *O. korrae* for each cultivar are detailed in Table 3. Of the 10 different bermudagrass cultivars, six of them were sampled at three locations or more: Latitude 36, Patriot, Riviera, TifSport, Tifway 419, and Vamont. *Ophiosphaerella herpotricha* was isolated > 58.6% more from Latitude 36, Patriot, and Riviera than Tifway 419 (Fig. 3). Moreover, *O. herpotricha*

was isolated from Latitude 36 63.9% and 75.3% more than from TifSport and Tifway 419, respectively. *Ophiosphaerella korrae* was isolated 70.1% more from Tifway 419 than from Patriot.

Discussion

Our data show that *O. herpotricha* and *O. korrae* are not uniformly distributed throughout the Mid-Atlantic United States, particularly Virginia. This differential geographic distribution could be derived from a number of contributing factors such as soil temperature, soil type, soil pH, soil nutrient content, bermudagrass cultivar, and inoculum source from either the plant material or naturally in the soil.

Ophiosphaerella herpotricha was more frequently isolated from the turfgrass facilities sampled in Delaware, the western region of Virginia, central North Carolina, and eastern Maryland than *O. korrae* in our study; however, only limited locations were sampled in these regions making inferences difficult. All of the aforementioned regions have generally cooler climates and soil temperatures than central Maryland, central Virginia, eastern North Carolina, and eastern Virginia where *O. korrae* was predominant. This suggests that soil temperature could influence the *Ophiosphaerella* species that primarily occurs in an area (Table 4). However, numerous studies have demonstrated that *O. herpotricha* and *O. korrae* grow at similar temperatures *in vitro* and colonize and damage bermudagrass at similar temperatures (Crahay et al., 1988; Perry et al., 2010; Tisserat et al., 1989; Walker and Smith, 1972; Walker et al., 2006). This suggests that soil temperature may not be a determinant of where *O. herpotricha* and *O. korrae* predominantly occur.

Soil type data was not collected in our study, yet SDS severity is greater in heavier soils than sandier soils (Pair et al., 1986). However, there has been no published literature on how soil type influences *O. herpotricha* and *O. korrae*. The piedmont and western regions of Virginia generally have clay loam soils while the eastern region of Virginia has more sandy loam soils,

which could potentially account for the difference in species distribution (Web Soil Survey, 2021). Soil pH was measured in our study at 30 of the locations, but there was no significant correlation between soil pH and the *Ophiosphaerella* species isolated ($P \geq 0.20$) (data not shown), yet previously published research suggests that pH affects *O. herpotricha* and *O. korrae* differently (Cottrill et al., 2016; Tredway et al., 2020). *Ophiosphaerella herpotricha* grows optimally *in vitro* at a pH range of 5 to 6 while *O. korrae* grows optimally at a pH of 6 (Cottrill et al., 2016). The relationship between *O. herpotricha* and *O. korrae* was not the same in the field, however, with a positive correlation existing between soil pH and SDS severity caused by *O. herpotricha* and a negative correlation between soil pH and SDS severity caused by *O. korrae* (Tredway et al., 2020). Although our data were not significant, the regression trends were similar between the *Ophiosphaerella* species and soil pH to what Tredway et al. (2020) observed. Soil pH could be a contributing factor to the non-uniform distribution of *O. herpotricha* and *O. korrae* throughout the Mid-Atlantic United States, but a more thorough investigation would need to be conducted.

Nutrient content data was not collected in our study, yet nutrient availability in the plant can influence *O. herpotricha* and *O. korrae* differently (Tredway et al., 2020). Tredway et al. (2020) demonstrated that foliar manganese content and severity of SDS caused by *O. korrae* were positively correlated and the opposite was true for *O. herpotricha*. Moreover, foliar calcium content and severity of SDS caused by *O. korrae* were inversely related (Tredway et al., 2020). Availability of manganese and calcium in the soil could be a determinant of the different geographic distribution of *O. herpotricha* and *O. korrae*, though more research is needed.

Ophiosphaerella herpotricha or *O. korrae* were detected more in certain bermudagrass cultivars (Fig. 3). There is a gradient of bermudagrass resistance or tolerance of different cultivars to SDS (Baird et al., 1998; Iriarte et al., 2005b; Martin et al., 2001a; Martin et al., 2001b; National

Turfgrass Evaluation Program, 2013; Pair et al., 1986; Tisserat et al., 1989). Moreover, *O. herpotricha* causes more severe SDS symptoms than *O. korrae* (Iriarte et al., 2005a). Iriarte et al. (2005b) showed that *O. herpotricha* caused greater SDS severity than *O. korrae* on the bermudagrass cultivar 'Tifgreen' while *O. herpotricha* and *O. korrae* caused similar SDS severity on the 'Midlawn,' 'Guymon,' and 'Champion' cultivars. This demonstrates that cultivars can vary in susceptibility, not only to SDS, but to the specific *Ophiosphaerella* species causing SDS. Variations in cultivar susceptibility could be a reason why either *O. herpotricha* or *O. korrae* was primarily isolated from certain cultivars in our study. Another reason could be that the pathogen came from sod farms at which different cultivars were grown. Many bermudagrass cultivars are vegetatively propagated making the sod farm a potential primary inoculum source (Hanna and Anderson, 2008). Parent material for certain cultivars came from the midwestern United States where *O. herpotricha* is the predominant SDS-causing species while parent material for other cultivars came from the southeastern United States where *O. korrae* is the primary SDS-causing species (Canegallo, 2016; Cottrill et al., 2016; Iriarte et al., 2004; Tredway et al., 2008; Wetzel et al., 1999). The sod source for the various cultivars could influence which *Ophiosphaerella* species is present at a location making it a potential contributing factor to the differential geographic distribution of *O. herpotricha* and *O. korrae* in the Mid-Atlantic United States. Species seem to follow patterns, but often both *O. herpotricha* and *O. korrae* are present at the same location further complicating management practices that are focused on a single species. More research needs to be conducted on how edaphic properties, cultivars, and other genetic and environmental factors influence the nonuniform distribution of *O. herpotricha* and *O. korrae*.

Acknowledgements

The authors thank the United States Department of Agriculture National Institute of Food and Agriculture for funding this work. The authors also thank Katie Dougherty, Camden Shelton, Alex Wong, and Scott Johnson for their assistance in sample collection and processing; all of the athletic field managers, golf course superintendents, and sod farmers that allowed us to sample their facilities; and Drs. Mike Goatley and Mizuho Nita for their guidance and support with this project.

References

Baird, J.H., Martin, D.L., Taliaferro, C.M., Payton, M.E., and Tisserat, N.A. 1998. Bermudagrass resistance to spring dead spot caused by *Ophiosphaerella herpotricha*. Plant Dis. 82:771-774.

Canegallo, A.L. 2016. Characterization and control of *Ophiosphaerella* spp. causing spring dead spot of bermudagrass in South Carolina, USA and Buenos Aires, Argentina. PhD diss. Clemson Univ., Clemson, SC.

Crahay, J.N., Dernoeden, P.H., and O'Neill, N.R. 1988. Growth and pathogenicity of *Leptosphaeria korrae* in bermudagrass. Plant Dis. 72:945-949.

Cottrill, D.J., Earlywine, D.T., and Miller, G.L. 2016. Assessment of nitrogen source, sulfur, and fall fungicide applications on the management of spring dead spot of bermudagrass. Plant Dis. 100:473-482. doi:http://dx.doi.org/10.1094/PDIS-05-15-0565-RE

Dernoeden, P.H., Crahay, J.N., and Davis, D.B. 1991. Spring dead spot and bermudagrass quality as influenced by nitrogen source and potassium. Crop Sci. 31:1674-1680.

Dunne, J.C., Tuong, T.D., Livingston, D.P., Reynolds, W.C., and Milla-Lewis, S.R. 2019. Field and laboratory evaluation of bermudagrass germplasm for cold hardiness and freezing tolerance. Crop Sci. 59:392-399. doi:10.2135/cropsci2017.11.0667.

Hanna, W.W. and Anderson, W.F. 2008. Development and impact of vegetative propagation in forage and turf bermudagrasses. Agron. J. 100:S-103-S-107. doi:10.2134/agronj2006.0302c.

Hutchens, W.J., Nagaoka, Y., Kerns, J.P., Goatley, J.M., Nita, M., and McCall, D.S. 2019. Variable Sensitivity of *Ophiosphaerella* spp. Causing Spring Dead Spot to Fungicides and Temperature. Abstract. *Crop Science Society of America* Annual Meeting, 2019.

Hutchens, W.J., Nagaoka, Y., Kerns, J.P., Goatley, J.M., Nita, M., Booth, J.C., and McCall, D.S. 2020. Differential Response of *Ophiosphaerella* Species *in situ* to Various Fungicides. Abstract. *Crop Science Society of America* Annual Meeting, 2020.

Iriarte, F.B. 2003. Genetic diversity and aggressiveness of *Ophiosphaerella korrae*, a cause of spring dead spot of bermudagrass. PhD diss. Kan. State Univ., Manhattan, KS.

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., and Tisserat, N.A. 2004. Genetic diversity and aggressiveness of *Ophiosphaerella korrae*, a cause of spring dead spot of bermudagrass. Plant Dis. 88:1341-1346.

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., Vincelli, P., Dixon, E.W., and Tisserat, N.A. 2005a. Aggressiveness of spring dead spot pathogens to bermudagrass. Int. Turf. Res. J. 10:258-264.

Iriarte, F.B., Fry, J.D., Martin, D.L., Todd, T.C., and Tisserat, N.A. 2005b. Effect of cold acclimation and freezing on spring dead spot severity in bermudagrass. HortScience 40:421-423.

Martin, D.L., Bell, G.E., Baird, J.H., Taliaferro, C.M., Tisserat, N.A., Kuzmic, R.M., Dobson, D.D., and Anderson, J.A. 2001a. Spring dead spot resistance and quality of seeded bermudagrasses under different mowing heights. Crop Sci. 41:451-456.

Martin, D.L., Bell, G.E., Taliaferro, C.M., Tisserat, N.A., Baird, J.H., Dobson, D.D., Kuzmic, R.M., and Anderson, J.A. 2001b. Spring dead spot resistance of inter-specific hybrid bermudagrasses. J. Intl. Turf. Res. 9:3-6.

Martinez, J.F.I., Flores, F.J., Koch, A.R., Garzon, C.D., and Walker, N.R. 2019. Multiplex end-point PCR for the detection of three species of *Ophiosphaerella* causing spring dead spot of bermudagrass. Plant Dis. 103:2010-2014. https.//doi.org/10.1094/PDIS-10-18-1727-RE

Martinez-Murillo, J.F., Hueso-Gonzalez, P., and Ruiz-Sinoga, J.D. 2017. Topsoil moisture mapping using geostatistical techniques under different Mediterranean climatic conditions. Sci. Total Environ. 595:400-412. https://doi.org/10.1016/j.scitotenv.2017.03.291

McCarty, L.B., Lucas, L.T., and DiPaola, J.M. 1992. Spring dead spot occurrence in bermudagrass following fungicide and nutrient applications. HortSci. 27.10:1092-1093.

National Turfgrass Evaluation Program. 2013. National Bermudagrass Test 2007-2012. USDA-ARS, Beltsville, MD.

Pair, J.C., Crowe, F.J., and Willis, W.G. 1986. Transmission of spring dead spot disease of bermudagrass by turf/soil cores. Plant Dis. 70:877-878.

Perry, D.H., Tomaso-Peterson, M., and Baird, R. 2010. Seasonal variation in frequency of isolation of *Ophiosphaerella korrae* from bermudagrass roots in Mississippi and pathogenicity and optimal growth of selected isolates. Mycologia 169:395-402. doi:10.1007/s11046-010-9273-x

Smith, A.M. 1971. Control of spring dead spot of couch grass turf in New South Wales. J. Sports Turf Res. Inst. 47:60-65.

Tisserat, N.A., Hulbert, S.H., and Sauer, K.M. 1994. Selective Amplification of rDNA Internal Transcribed Spacer Regions to Detect *Ophiosphaerella korrae* and *O. herpotricha*. Phytopathology 84:478-482.

Tisserat, N.A., Pair, J.C., and Nus, A. 1989. *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermudagrass in Kansas. Plant Dis. 73:933-937.

Tredway, L.P., Butler, E.L., Soika, M.D., and Bunting, M.L. 2008. Etiology and management of spring dead spot of hybrid bermudagrass in North Carolina, USA. Acta Hort. 783:535-546.

Tredway, L.P., Soika, M.D., Butler, E.L., and Kerns, J.P. 2020. Impact of nitrogen source, fall fertilizers, and preventative fungicides on spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha*. Crop Sci. Special Issue: International Turfgrass Research Conference: 1-10. doi:10.1002/csc2.20306

Walker, J. and Smith, A.M. 1972. *Leptosphaeria narmari* and *L. korrae* spp. nov., two long-spored pathogens of grasses in Australia. Trans. Br. Mycol. Soc. 58:459-466.

Walker, N.R., Mitchell, T.K., Morton, A.N., and Marek, S.M. 2006. Influence of temperature and time of year on colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. Plant Dis. 90:1326-1330.

Web Soil Survey. 2021. Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Available online at the following link: http://websoilsurvey.sc.egov.usda.gov/. Accessed [22 Apr 2021].

Wetzel, H.C., III, Skinner, D.Z., and Tisserat, N.A. 1999. Geographic distribution and genetic diversity of three *Ophiosphaerella* species that cause spring dead spot of bermudagrass. Plant Dis. 83:1160-1166.

Younger, V.B., Gibeault, V.A., and Breece, J.R. 1972. Turf bermudagrass. California Turfgrass Culture 22:1-3.

Table 1. Primer pairs used for amplifying *O. herpotricha* or *O. korrae* in qPCR reactions (Martinez et al., 2019; Tisserat et al., 1994). All primers were purchased from Thermo Fisher Scientific [Waltham, MA].

Species	Primer Name	Primer Sequence
O. herpotricha forward	OHITS1	5'– CCAAGTGTAGAACAAACTACGC – 3'
O. herpotricha reverse	OHITS2	5' – AAAAGGCTTATTGGGTGCCTAT – 3'
O. herpotricha forward	OHERFW	5' – CGTAATCTCCAAAGATGGCCAA – 3'
O. herpotricha reverse	OHERRV	5' – CACGCAGTTGGTAGAAACGT – 3'
O. korrae forward	OKITS1	5' – CCAAGTGCAGCACAAACTGCATG – 3'
O. korrae reverse	OKITS2	5' – AAGAGGCTTAATGGGTGCCCAC – 3'
O. korrae forward	OKORFW	5' – GGACACCCCATTGAACCTWTTT– 3'
O. korrae reverse	OKORRV	5' – GTTATCWGACGCAGTGGAGTG – 3'

Table 2. Detection frequency (0 to 1) of *O. herpotricha* and *O. korrae* from SDS symptomatic bermudagrass or zoysiagrass from 51 locations (golf courses, athletic fields, or sod farms) across Delaware, Maryland, North Carolina, and Virginia.

City	State	Cultivar	No. of Samples	*O. herpotricha*	*O. korrae*
Dover	DE	Patriot	6	0.67	0
Newark	DE	Latitude 36	4	1	0.75
College Park (site 1)	MD	Latitude 36/Vamont	4	0.75	0
College Park (site 2)	MD	Latitude 36	8	0.88	0.25
Easton (site 1)	MD	Patriot	10	0.20	0
Easton (site 2)	MD	Latitude 36	6	1	0
Grasonville	MD	Latitude 36	2	1	0
Queenstown	MD	Patriot	14	0.64	0.14
Rockville	MD	Patriot	6	0.33	0.50
Salisbury	MD	Patriot	6	0.83	0
Stevensville	MD	Patriot	4	0.50	0.50
Charlotte	NC	Tifway 419	4	0	1
Greensboro	NC	Tifway 419	4	0.75	0.25
Merry Hill	NC	TifSport	4	0	1
Amherst	VA	Common	4	1	0
Appomattox	VA	Riviera	4	1	0
Blacksburg	VA	Patriot	10	0.70	0.20
Blackstone	VA	TifEagle	10	0.60	0.10
Cape Charles	VA	TifSport	14	0	0.29
Charlottesville	VA	Vamont	10	0.70	0.10
Culpeper	VA	Riviera	6	0.83	0
Danville	VA	Norman/Common	4	1	0.25
Emporia	VA	Common	10	0.80	0.10
Front Royal (site 1)	VA	N/A	4	0	0.50
Front Royal (site 2)	VA	Riviera	4	0.50	0.75
Fredericksburg	VA	Vamont	4	0.75	0.50
Halifax	VA	Common	8	0.75	0.25
Harrisonburg	VA	Patriot	1	1	0
Lawrenceville	VA	Common	4	0	0.50
Manakin-Sabot	VA	Vamont	4	0	1
Martinsville	VA	Common/Vamont	8	0.75	0
Midlothian	VA	Tifway 419	16	0.81	0.13
Mineral	VA	Patriot	4	0	0.75
Newport News	VA	Celebration	4	0.75	0
Norfolk (site 1)	VA	TifSport	4	1	0
Norfolk (site 2)	VA	N/A	10	0.60	0.50
North Chesterfield	VA	Tifway 419	4	0	1
Palmyra	VA	Vamont	4	1	0.25
Petersburg	VA	Vamont	9	0.33	0.78
Portsmouth	VA	Common	6	1	0.17
Richmond	VA	TifSport	8	0.38	0.63
Roanoke	VA	N/A	8	1	0.25
Rocky Mount	VA	Patriot	4	0.75	0
Ruther Glen (site 1)	VA	N/A	4	0.25	1
Ruther Glen (site 2)	VA	Empire Zoysiagrass	10	0.20	0.80
Stuarts Draft	VA	Patriot	8	0.75	0.13
Virginia Beach (site 1)	VA	Tifway 419	4	0	1
Virginia Beach (site 2)	VA	Tifway 419	4	0	1
Williamsburg (site 1)	VA	Tifway 419	8	0.38	0.50
Williamsburg (site 2)	VA	Tifway 419	6	0	0.50
Winchester	VA	N/A	4	0	0.75

Table 3. Detection frequency (0 to 1) of *O. herpotricha* and *O. korrae* from different SDS symptomatic bermudagrass or zoysiagrass cultivars.

Cultivar	**Location of Parent Material[1]**	**No. of Locations with Cultivar**	**No. of Samples**	***O. herpotricha***	***O. korrae***
Celebration	Australia	1	4	0.75	0
Common	N/A	5	32	0.75	0.19
Common/Norman	N/A	1	4	1	0.25
Common/Vamont	N/A	1	8	0.75	0
Latitude 36	Oklahoma	4	20	0.95	0.25
Latitude 36/Vamont	Oklahoma/Virginia	1	4	0.75	0
Patriot	Oklahoma	11	73	0.56	0.18
Riviera	Oklahoma	3	14	0.79	0.21
TifEagle	Georgia	1	10	0.60	0.10
TifSport	Georgia	4	30	0.23	0.43
Tifway 419	Georgia	8	50	0.38	0.52
Vamont	Virginia	5	31	0.55	0.48
Empire Zoysia	Brazil	1	10	0.20	0.80
Unknown	N/A	5	30	0.50	0.53

[1]Location where the cultivar was developed.

Table 4. Mean annual high and low temperatures for eight cities in which turfgrass facilities were sampled within. Selected cities range over a variety of geographically and environmentally unique areas within the Mid-Atlantic United States. The likely *Ophiosphaerella* species to occur at each location is based on the heat map (Fig. 2) generated from isolation frequency data.

Location	**Likely *Ophiosphaerella* spp.**	**Mean Annual High Temp. (°C)**	**Mean Annual Low Temp. (°C)**
Dover, DE	*O. herpotricha*	18.9	8.3
College Park, MD	Mixed	18.9	8.3
Salisbury, MD	Mixed	18.9	7.8
Greensboro, NC	*O. herpotricha*	20.6	9.4
Blacksburg, VA	*O. herpotricha*	17.2	4.4
Richmond, VA	*O. korrae*	21.1	8.9
Virginia Beach, VA	*O. korrae*	20.6	11.1
Williamsburg, VA	*O. korrae*	20.0	9.4

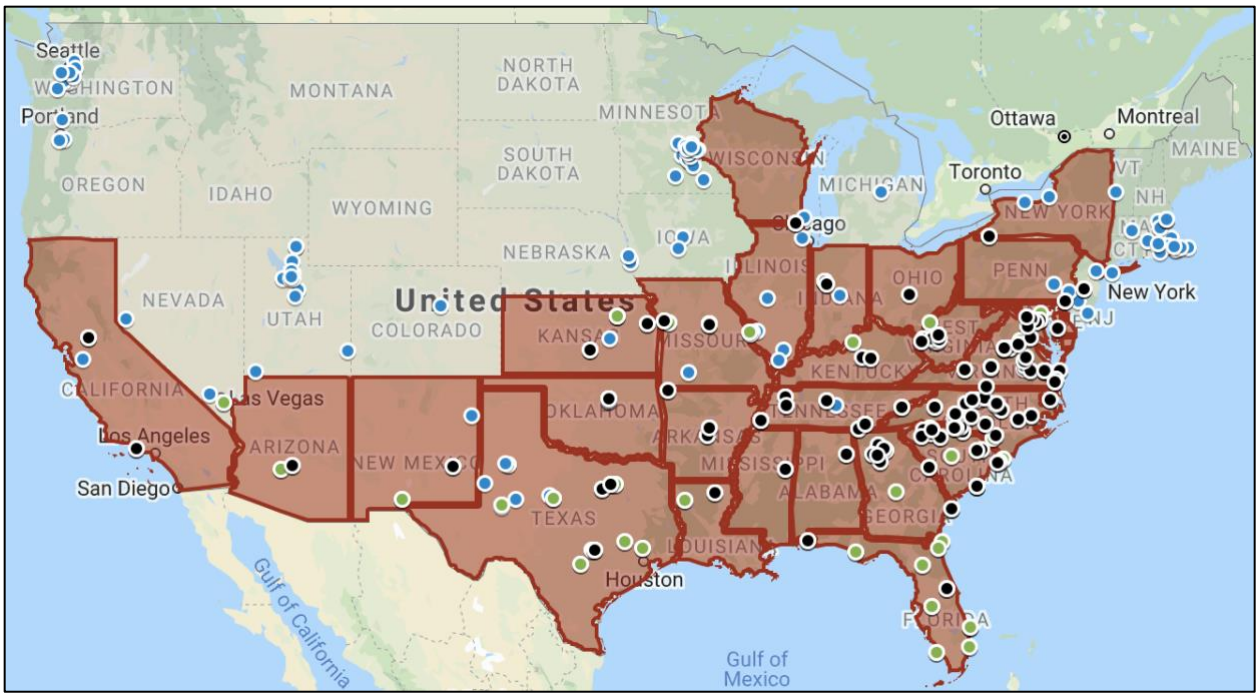

Figure 1. Turfgrass managers from states highlighted in red (27 total) reported SDS on bermudagrass in an online survey (blue dots = no bermudagrass; green dots = bermudagrass - SDS; black dots = bermudagrass + SDS).

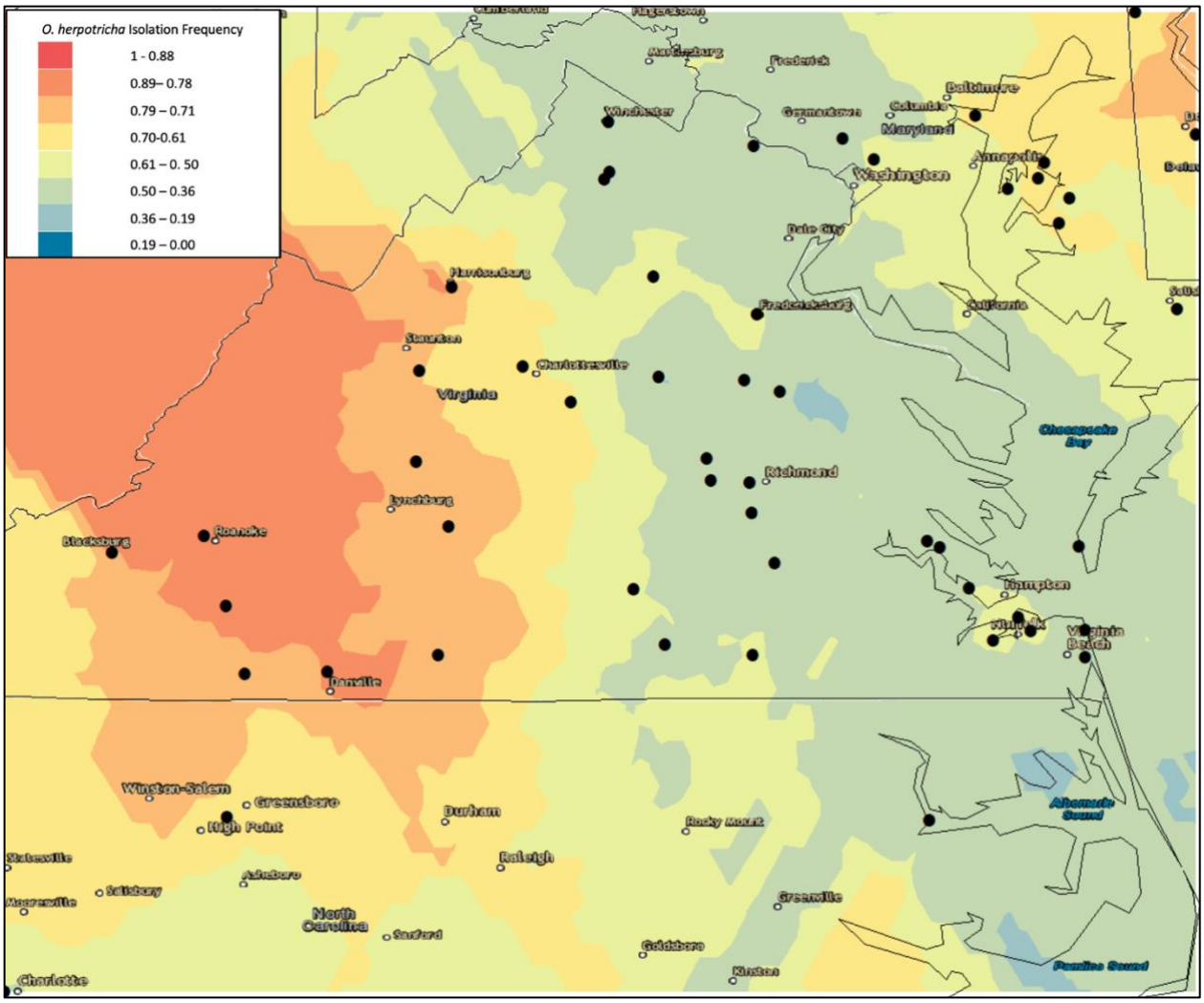

Figure 2. Cokriged geographic distribution heat map of *O. herpotricha*, considering *O. korrae* as a covariate in the Mid-Atlantic United States. There were eight different classes used in the cokriging analysis. The *O. herpotricha* isolation frequency was classified using the geometric interval classification method.

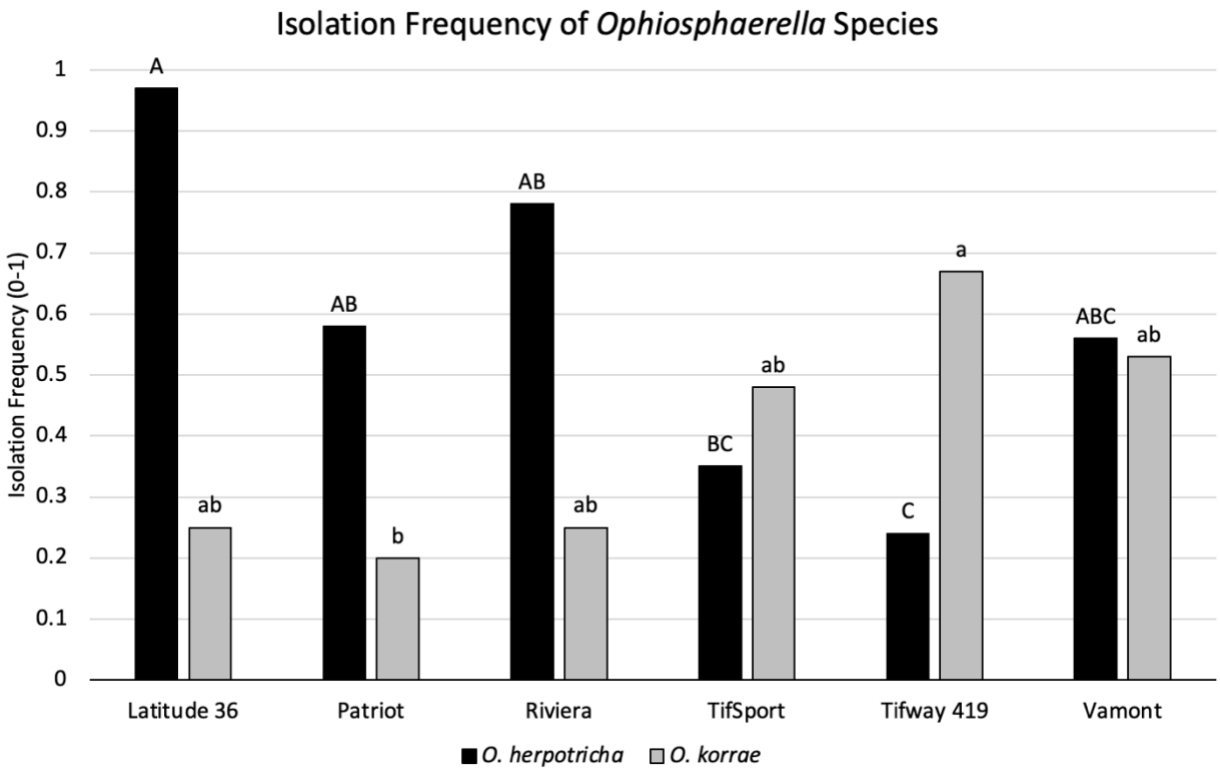

Figure 3. Isolation frequency of *Ophiosphaerella* species. Black bars represent *O. herpotricha* and gray bars represent *O. korrae*. Means are compared within *Ophiosphaerella* species across cultivars using the Student's *t*-test. Bars of the same color with different letters are significantly different ($P < 0.1$).

Chapter 3: Differential Responses of *Ophiosphaerella herpotricha* and *O. korrae* to Fungicides *In Vitro* and *In Situ*

Abstract

Ophiosphaerella herpotricha and *O. korrae* are the two most common fungal species that cause the bermudagrass disease spring dead spot (SDS) in North America. Previous research shows that they differ in aggressiveness, response to fertilizer type, ability to grow at certain pH ranges, and sensitivity to certain fungicides. The purpose of this project was to determine how *O. herpotricha* and *O. korrae* respond *in vitro* and *in situ* to various fungicides and fungicide groups. An *in vitro* fungicide sensitivity assay was conducted on four *O. herpotricha* isolates and four *O. korrae* isolates testing 13 different fungicides belonging to three different fungicide groups: (DMIs: fenarimol, mefentrifluconazole, myclobutanil, propiconazole, tebuconazole; QoIs: azoxystrobin, fluoxastrobin, pyraclostrobin; SDHIs: fluopyram, fluxapyroxad, isofetamid, penthiopyrad, and pydiflumetofen) at six different concentrations (0, 0.001, 0.01, 0.1, 1, or 10 $\mu g\ mL^{-1}$). An *in situ* fungicide screening tested the same fungicides as the *in vitro* study with the exception of fenarimol and fluopyram. This trial was conducted at locations containing either a predominantly *O. herpotricha*, *O. korrae*, or mixed *Ophiosphaerella* population. Fungicides were generally less effective against *O. korrae* than *O. herpotricha in vitro* and *in situ*. Moreover, the SDHIs were the most efficacious group of fungicides against SDS, regardless of *Ophiosphaerella* species. Isofetamid and pydiflumetofen had the lowest EC_{50} values *in vitro* and generally greatest SDS suppression *in situ*. This research demonstrates that fungicide selection for SDS should be based on the *Ophiosphaerella* species present, or fungicides should be applied that can suppress both *O. herpotricha* and *O. korrae*.

Introduction

Spring dead spot (SDS), caused by *Ophiosphaerella* spp., is a detrimental disease to bermudagrass (*Cynodon dactylon* L.) that experiences winter dormancy when grown in suboptimal regions of adaptation. The pathogens infect roots, rhizomes, and stolons primarily in the autumn months weakening the bermudagrass going into winter dormancy (Perry et al., 2010; Walker et al., 2006). Sub-freezing temperatures can damage bermudagrass infected with *Ophiosphaerella* spp. and cause isolated necrotic patches at spring green-up (Wadsworth and Young, 1960). The presence of the disease causes aesthetic, playability, and safety issues to bermudagrass golf courses, athletic fields, and home lawns (Martin et al., 2001).

Spring dead spot is caused by three different *Ophiosphaerella* species: *O. herpotricha* (Fr:Fr) J. Walker, *O. korrae* (J. Walker & A.M. Smith) Shoemaker & C.E. Babcock (=*Leptosphaeria korrae* J. Walker & A.M. Smith), and *O. narmari* (J. Walker & A.M. Smith) Wetzel, Hulbert, & Tisserat (=*L. narmari* J. Walker & A.M. Smith) (Flores et al., 2017; Iriarte et al., 2004; Iriarte et al., 2005a; Wetzel et al., 1999). The two predominant species that cause SDS in North America are *O. herpotricha* and *O. korrae* while *O. narmari* is more commonly found in Australia and New Zealand (Cottrill et al., 2016; Flores et al., 2017; Hawkes, 1987; Hutchens et al., 2021; Iriarte et al., 2004; Tisserat et al., 2004; Tredway et al., 2008; Walker and Smith, 1972; Wetzel et al., 1999). North American isolates of *O. herpotricha* and *O. korrae* are part of a monophyletic lineage and species correspond within well-supported clades in the *Ophiosphaerella* genus (Flores et al., 2017). Although these two species are closely related, it is well documented that *O. herpotricha* is more aggressive than *O. korrae* by causing greater SDS severity and incidence in inoculated plots (Iriarte, 2003; Iriarte et al., 2005a; Iriarte et al., 2005b; Tredway et

al., 2008; Tredway et al., 2020). Furthermore, they differ in their response to both cultural and chemical management practices (Cottrill et al., 2016; Tredway et al., 2020).

Ophiosphaerella herpotricha and *O. korrae* respond differently to a variety of cultural management practices (Cottrill et al., 2016; Tredway et al., 2020). Cottrill et al. (2016) showed that *O. korrae* grew optimally at a pH of 6 in artificial media while *O. herpotricha* grew optimally at a pH range of 5 to 6. Tredway et al. (2020) found a positive correlation with soil pH and SDS caused by *O. herpotricha* and a negative correlation with soil pH and SDS caused by *O. korrae*, so management practices that influence soil pH could affect SDS incidence and severity depending on the *Ophiosphaerella* species present. The authors also determined that calcium nitrate was effective at suppressing *O. korrae* while ammonium sulfate was effective at suppressing *O. herpotricha* in a field study (Tredway et al., 2020).

The response of SDS to fungicides is highly variable. The fungicides azoxystrobin, myclobutanil, propiconazole, and fenarimol suppressed SDS in a 2003 study, yet fenarimol provided greater SDS suppression the following year (Tredway et al, 2008). Tebuconazole did not suppress SDS in a 2012 study, yet it suppressed SDS by 49% compared to a nontreated control the following year (Earlywine and Miller, 2012; Earlywine and Miller, 2013). Penthiopyrad applied twice at 2.1 kg ha^{-1} in the fall has repeatedly reduced SDS patch area and severity, yet in a 2017-2018 study the fungicide did not reduce SDS area under the disease progress curve (AUDPC) compared to a nontreated control (Earlywine and Miller, 2013; Freund et al., 2019; Galle et al., 2019; Stephens et al., 2020). The inconsistency in fungicide efficacy could be attributed to the different species that cause SDS. Tredway et al. (2020) showed that fenarimol, myclobutanil, propiconazole, propiconazole + azoxystrobin, and tebuconazole all suppressed SDS caused by *O. herpotricha* and did not suppress SDS caused by *O. korrae*.

Fungicides belonging to the demethylase inhibitors (DMIs), quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs) suppress SDS (Stephens et al., 2020). Our goal was to screen numerous fungicides belonging to the DMIs, QoIs, and SDHIs against *O. herpotricha* and *O. korrae* isolates *in vitro* and *in situ* against SDS at locations with predominant *O. herpotricha*, *O. korrae*, and mixed *O. herpotricha* and *O. korrae* populations.

Materials and Methods

In vitro fungicide sensitivity assay

Four *O. herpotricha* and four *O. korrae* isolates were obtained from the North Carolina State University Turfgrass Pathology Lab. Isolates were stored on filter paper fragments in a -80°C freezer and plated onto potato dextrose agar (PDA) (39 g L^{-1}) [Difco Laboratories Inc., Franklin Lakes, NJ, USA] amended with 50 μg mL^{-1} of chloramphenicol, streptomycin sulphate, and tetracycline (PDA^{+++}) (Rioux et al., 2014). Isolates were subsequently sub-cultured onto non-amended PDA and grown free of contaminants. DNA was then extracted from each isolate culture and polymerase chain reaction (PCR) was conducted using the OHITS1, OHITS2, OKITS1, and OKITS2 primers developed by Tisserat et al. (1994) to confirm the *Ophiosphaerella* species. The species of each isolate that was not confirmed in the original PCR run was later confirmed by real-time PCR with the OHERFW, OHERRV, OKORFW, OKORRV primers (Martinez et al. 2019).

The *in vitro* fungicide sensitivity assay included 13 different fungicides belonging to three different fungicide groups (Table 1). Salicylhydroxamic acid (SHAM) was not included with the QoI fungicides in our study per the findings of Liang et al. (2019). Three mm plugs of each *O. herpotricha* and *O. korrae* isolate were placed onto the center of 100 mm PDA plates amended with six concentrations of each fungicide (0, 0.001, 0.01, 0.1, 1, and 10 μg mL^{-1}). The isolates were incubated in the dark at 20°C for 14 days. After incubation, mycelial diameters were

measured in two directions. There were three replications, and the study was repeated. Both runs were combined to calculate the EC_{50} values using the PROC REG procedure in SAS (Statistical Analysis Software, Version 9.4, SAS Institute, Cary, NC) based on the percent mycelial inhibition compared to the nontreated control following the methods of Hutchens et al. (2019). EC_{50} values were then pooled across isolates within species, subjected to analysis of variance (ANOVA), and means were separated using Fisher's Protected LSD test ($P < 0.1$).

In situ fungicide screening

A fungicide screening was also conducted *in situ*. Field studies were initiated with fungicide applications in the fall of 2019, and data were collected in the spring and summer of 2020. The study was repeated the following year with fungicide applications initiated in the fall of 2020 and data collected in the spring and summer of 2021. The study was conducted at six different locations throughout Virginia and Maryland. One location had a predominantly *O. korrae* population, three locations had a predominantly *O. herpotricha* population, and one location had a mixture of *O. herpotricha* and *O. korrae* (Table 2). The remaining two locations were artificially inoculated at a 5-cm depth at three points in each plot with 50 cc of oats colonized with either *O. herpotricha*, *O. korrae*, or both species (Table 2). Inoculations were performed following the methods of Iriarte et al. (2005). Symptoms from inoculated plots did not appear at either location for the duration of the study.

Studies were arranged as randomized complete block designs (RCBD) with four replications and 23 treatments. Fungicides were applied either once at full label rate or twice at the half rate three to four weeks apart. Treatments are highlighted in Table 3. All fungicides were applied with a CO_2-pressurized sprayer delivering solution at 276 kPa of pressure with a water carrier volume of 842 L ha^{-1}. Fungicides were applied in the fall when five-day average soil

temperatures at a 0 to 10 cm depth were between 10.7 and 22.1°C, as estimated using greencastonline.com (Syngenta, Basel, Switzerland). Fungicide applications were immediately irrigated with 0.6 cm of post-application irrigation (PAI) through hand-watering of individual plots. In 2019 the Culpeper, VA location was irrigated with only 0.01 cm of PAI due to irrigation system issues, and the second application at Palmyra, VA was irrigated with only 0.34 cm of PAI due to saturated soils from previous rainfall only holding 0.34 cm of supplemental irrigation.

Data were collected beginning after spring greenup in the spring/summer of 2020 and the spring/summer of 2021. Three assessments were conducted each year at two to four weeks apart. Patch count and percent spring dead spot were measured on each assessment date. Patch count and percent spring dead spot data were transformed to area under the disease progress curve (AUDPC) to encompass change in disease over time. The treatments were pooled together by number of fungicide applications, and data from both location and year were analyzed separately, subjected to ANOVA, and means were separated using a Student's *t*-test ($P < 0.1$) in JMP Pro 15 [SAS Institute, Cary, NC]. With one and two fungicide applications being pooled, each block had an unequal number of replications with the nontreated control having one replication per block and each fungicide having two replications per block. Moreover, the AUDPC data for the different fungicides were also pooled together by fungicide group, and data were subjected to ANOVA and means were separated using a Student's *t*-test ($P < 0.1$) in JMP Pro 15 [SAS Institute, Cary, NC]. This also led to unequal replications within each block with the nontreated control having one replication per block, the DMIs having eight replications per block, the QoIs having six replications per block, and the SDHIs having eight replications per block.

Results

In vitro fungicide sensitivity assay

The EC_{50} values varied across isolates, fungicides, and fungicide groups (Table 1). Within the DMIs, propiconazole had the lowest average EC_{50} value (0.15 mg L^{-1}) across both *O. herpotricha* and *O. korrae* isolates. Within the QoIs, pyraclostrobin had the lowest average EC_{50} value of 0.12 mg L^{-1} across all isolates. Moreover, pydiflumetofen suppressed all *Ophiosphaerella* isolates more than the other SDHI fungicides with an average EC_{50} value of 0.02 mg L^{-1}. Additionally, there was variability in how well certain fungicides suppressed *O. herpotricha* versus *O. korrae* isolates. *Ophiosphaerella korrae* EC_{50} values were $\leq$ 0.13 mg L^{-1} for mefentrifluconazole, yet EC_{50} values for *O. herpotricha* isolates were $\geq$ 0.78 mg L^{-1}. In contrast, *O. herpotricha* EC_{50} values were $\leq$ 2.78 mg L^{-1} and 2.17 mg L^{-1} for azoxystrobin and fluoxastrobin, respectively; however, three of the four *O. korrae* isolates had EC_{50} values > 10 mg L^{-1} for azoxystrobin and all four *O. korrae* isolates had EC_{50} values > 10 mg L^{-1} for fluoxastrobin.

When EC_{50} values for *O. herpotricha* and *O. korrae* isolates were pooled by *Ophiosphaerella* species, fenarimol, myclobutanil, propiconazole, pyraclostrobin, fluxapyroxad, and penthiopyrad differentially suppressed *O. herpotricha* and *O. korrae* (Table 4). The EC_{50} values for *O. korrae* were 43.9% lower than *O. herpotricha* EC_{50} values for fenarimol. Fenarimol was the only fungicide to suppress *O. korrae* more than *O. herpotricha in vitro*. Myclobutanil, propiconazole, pyraclostrobin, fluxapyroxad, and penthiopyrad produced 49.2, 55.0, 68.4, 64.0, and 69.2% lower EC_{50} values, respectively, for *O. herpotricha* than *O. korrae*.

In situ fungicide screening

Disease pressure varied across locations and years. The greatest SDS incidence (patch number) within a single plot on any rating date at any location in both 2020 and 2021 was at the Cape Charles, VA (*O. korrae*) location with up to 22 patches in a single plot in 2020 and up to 28 patches in a single plot in 2021 (data not shown). The lowest disease incidence in both 2020 and

2021 was at the Salisbury, MD (*O. herpotricha*) location with a maximum of 12 patches in a single plot in 2020 and a maximum of 16 patches in a single plot in 2021 (data not shown). A similar trend existed with the greatest SDS severity (percent SDS). The maximum percent SDS documented in a single plot on any rating date across locations was at the Cape Charles, VA location for both 2020 and 2021, with up to 30% SDS in a single plot in 2020 and up to 45% SDS in a single plot in 2021 (data not shown). However, the locations with the lowest maximum disease severity in a single plot differed between years. The Palmyra, VA (both *O. herpotricha* and *O. korrae* present) location had a maximum of 10% SDS in a single plot in 2020 and the Culpeper, VA (*O. herpotricha*) location had a maximum of 21% SDS in a single plot in 2021 making them the locations with the lowest maximum disease severity in a single plot (data not shown).

One application versus two applications of fungicide across all locations and years pooled together was not significant for patch number AUDPC ($P = 0.5364$) or percent SDS AUDPC ($P = 0.8275$) —the nontreated control was removed from analysis when comparing one and two fungicide applications and their interactions. Moreover, interactions between fungicide and number of fungicide applications across all locations and years pooled together was not significant for patch number AUDPC ($P = 0.7832$) or percent SDS AUDPC ($P = 0.7559$). Two location by year combinations did have a significant main effect of application number ($P < 0.1$) on patch number AUDPC. Two fungicide applications resulted in 21% lower patch number AUDPC at Palmyra, VA (both *O. herpotricha* and *O. korrae* present) in 2020 ($P = 0.0756$), and two fungicide applications resulted in 24% lower patch number AUDPC at Salisbury, MD (*O. herpotricha*) in 2021 ($P = 0.0827$). However, fungicide was the most significant effect for every location by year in which there was a significant main effect of application number. Therefore, the main effect of fungicide is what will be discussed in this manuscript.

Patch number AUDPC was numerically the highest at Cape Charles, VA (*O. korrae*) in 2020 (Table 5). Fungicides varied in their efficacy at different locations depending on disease pressure and *Ophiosphaerella* species. Propiconazole, for example, suppressed SDS by ≥ 70.7% at Blacksburg, VA (inoculated) and Salisbury, MD (*O. herpotricha*), yet the fungicide did not suppress SDS at any other location (Table 5). Most fungicides did not suppress SDS at the location infested with only *O. korrae* (Cape Charles, VA). Only mefentrifluconazole and isofetamid reduced patch number AUDPC (≥ 77.2%) compared to the nontreated control at Cape Charles, VA. Moreover, isofetamid provided the greatest patch number AUDPC reduction at all locations with a significant fungicide effect in 2020.

In 2021, at Culpeper, VA (*O. herpotricha*), no fungicide reduced patch number AUDPC compared to the nontreated control, but fungicides reduced patch number AUDPC at all other locations. Like 2020, only mefentrifluconazole and isofetamid reduced patch number AUDPC (≥ 76.8%) compared to the nontreated control at Cape Charles, VA (*O. korrae*). Isofetamid and mefentrifluconazole reduced patch number AUDPC at all locations similarly except for Palmyra, VA (both *O. herpotricha* and *O. korrae* present). Isofetamid was among the most efficacious fungicides at all locations. Moreover, isofetamid and pydiflumetofen resulted in similar patch number AUDPC values at all locations highlighting that the two fungicides are similarly efficacious. Penthiopyrad also reduced patch number AUDPC similarly to isofetamid at all locations infested by *O. herpotricha* or a mixed population—only at Cape Charles, VA did isofetamid reduce patch number AUDPC more than penthiopyrad.

Fungicide effect on percent SDS AUDPC was observed only at Salisbury, MD (*O. herpotricha*), Culpeper, VA (*O. herpotricha*), and Palmyra, VA (both *O. herpotricha* and *O. korrae* present) in 2020 (Table 7). No fungicides reduced percent SDS AUDPC compared to the

nontreated control at Salisbury, MD (*O. herpotricha*) in 2020. At Culpeper, VA (*O. herpotricha*) only mefentrifluconazole provided significant reduction of percent SDS AUDPC compared to the nontreated control, yet at Palmyra, VA (both *O. herpotricha* and *O. korrae* present) isofetamid, penthiopyrad, and pydiflumetofen were the most efficacious fungicides providing ≥ 91.8% suppression of SDS.

Different from 2020, fungicide effects on percent SDS AUDPC were observed at all locations but Culpeper, VA (*O. herpotricha*) in 2021 (Table 8). No fungicides reduced percent SDS AUDPC compared to the nontreated control at Cape Charles, VA (*O. korrae*), but mefentrifluconazole and isofetamid had the numerically lowest percent SDS AUDPC. Mefentrifluconazole, isofetamid, penthiopyrad, and pydiflumetofen had similar percent SDS AUDPCs at all locations and generally provided the greatest disease suppression. Isofetamid, penthiopyrad, and pydiflumetofen were the only fungicides to reduce percent SDS AUDPC (≤ 76.4%) at Blacksburg, VA (inoculated), Salisbury, MD (*O. herpotricha*), and Palmyra, VA (both *O. herpotricha* and *O. korrae* present) compared to the nontreated control suggesting that these three fungicides are the most consistent and efficacious against SDS.

Generally, the SDHI fungicide group was the most efficacious against SDS. The SDHIs had the lowest amount of disease for four of 12 site-years with no other fungicide group reducing SDS significantly more than the SDHIs for any site-year. In 2020, the SDHIs reduced patch number AUDPC similarly to the DMIs and QoIs at Blacksburg, VA (inoculated) (Fig. 1a). At Salisbury, MD (*O. herpotricha*) the SDHIs and DMIs had similar patch number AUDPCs to the SDHIs reducing patch number AUDPC by 61.7 % compared to the QoIs. At Culpeper, VA (*O. herpotricha*) the SDHIs and DMIs, once again, provided similar patch number AUDPC, yet the DMIs were the only group of fungicides to suppress SDS compared to the nontreated control

providing 52.8% suppression. Only the SDHIs reduced patch number AUDPC at Cape Charles, VA (*O. korrae*) (58.4% reduction), but the DMIs produced similar patch number AUDPC to the SDHIs. At Palmyra, VA (both *O. herpotricha* and *O. korrae* present) the SDHIs reduced patch number AUDPC by 56.8, 68.3, and 78.9% compared to the QoIs, DMIs, and nontreated control, respectively. In 2021, the SDHIs reduced patch number AUDPC by ≥ 45.8% compared to the other fungicide groups and the nontreated control at all locations except for Culpeper, VA (*O. herpotricha*) and Cape Charles, VA (*O. korrae*) (Fig. 1b). The DMIs reduced patch number AUDPC by ≥ 32.5% compared to the QoIs at Blacksburg, VA (inoculated) and Cape Charles, VA (*O. korrae*). Otherwise, the DMIs and QoIs had similar patch number AUDPCs.

The only location in which fungicide group reduced percent SDS AUDPC compared to the nontreated control in 2020 was Palmyra, VA (both *O. herpotricha* and *O. korrae* present) (Fig. 2a). At this location the DMIs, QoIs, and SDHIs reduced percent SDS AUDPC by ≥ 44.9% compared to the nontreated control. Moreover, the SDHIs reduced percent SDS AUDPC by ≥ 56.2% compared to the DMIs and QoIs. Similar differences existed at Palmyra, VA (both *O. herpotricha* and *O. korrae* present) in 2021 as well (Fig. 2b). Additionally, the SDHIs reduced percent SDS AUDPC by ≥ 63.1% compared to all other fungicide groups at Blacksburg, VA (inoculated) and Salisbury, MD (*O. herpotricha*) in 2021.

Discussion

The data from both the *in vitro* studies suggest that *O. herpotricha* and *O. korrae* respond differently to certain fungicides, which is similar to what was previously reported in field studies (Tredway et al., 2020). Moreover, SDS tended to be differentially suppressed in the field depending on the *Ophiosphaerella* population at that location. There has been no reported *in vitro* fungicide sensitivity screening for *O. herpotricha* and *O. korrae*. The *in vitro* experiment

demonstrated that *O. korrae* and *O. herpotricha* isolates vary in their sensitivity to many fungicides. The different ectotrophic root-infecting fungal species that cause take-all root rot on bermudagrass have also been shown to have numerically different EC_{50} values to fungicides *in vitro* (Stephens., 2021). The *O. korrae* isolates we tested were less sensitive to many fungicides than *O. herpotricha* isolates except for fenarimol in which the phenomenon was reversed. This trend was also observed in our field studies with only mefentrifluconazole and isofetamid consistently suppressing SDS at the *O. korrae* site (Cape Charles, VA). In contrast, many fungicides suppressed SDS at locations infested with *O. herpotricha* or a mixed population. Tredway et al. (2020) also observed greater fungicidal suppression of SDS caused by *O. herpotricha* than *O. korrae*. The reasons for the different responses of *O. herpotricha* and *O. korrae* to fungicides is not known and warrants further investigation.

There was inconsistency in the efficacy of many fungicides between locations and years, which is in line with what other researchers have observed (Booth et al., 2021; Earlywine and Miller, 2012; Earlywine and Miller, 2013; Freund et al., 2019; Galle et al., 2019; Stephens et al., 2020; Tredway et al, 2008). The different *Ophiosphaerella* species, the timing of the fungicide applications, severity of the winter, inability to irrigate the fungicide in with enough water at Culpeper, VA in 2019, and unexplained variation could all be reasons for the inconsistency in our field studies (Booth et al., 2021; Hutchens et al., 2019; Tredway et al., 2020; Walker, 2009).

Generally, the number of fungicide applications did not significantly affect SDS suppression, but the fungicide generally did influence SDS suppression. Mefentrifluconazole and isofetamid were consistently efficacious fungicides against SDS, regardless of *Ophiosphaerella* species. Penthiopyrad and pydiflumetofen were highly suppressive of SDS at all locations except for the *O. korrae* location (Cape Charles, VA) which, for penthiopyrad, was similar to what we

observed in our *in vitro* screening. Moreover, mefentrifluconazole was highly effective against *O. korrae* in the field, comparable with the *in vitro* results. Lastly, isofetamid was highly efficacious against *Ophiosphaerella* in both the field and lab studies. Isofetamid has also previously been documented as efficacious in field testing trials (Freund et al., 2019; Stephens et al., 2020). Isofetamid may be highly effective against SDS in the field, in part, due to its moderate soil adsorption coefficient (K_{oc}) of 281-615 potentially allowing for more fungicide to reach the basal and underground portions of the plant where *Ophiosphaerella* spp. infect (Latin, 2021).

The SDHIs have been documented to be effective against SDS (Booth et al., 2021; Freund et al., 2019; Roberson et al., 2017; Stephens et al., 2020). We observed that the SDHIs consistently provided excellent SDS suppression, particularly isofetamid, penthiopyrad, and pydiflumetofen. We speculate that *Ophiosphaerella* spp. may be more sensitive to the SDHIs due to their mode of action, which targets succinate dehydrogenase a crucial enzyme necessary for mitochondrial respiration, yet more research needs to be done to elucidate why the SDHIs are highly efficacious against SDS (Latin, 2021). Most of the DMIs were moderately efficacious against SDS, however, mefentrifluconazole was consistently highly efficacious against SDS in our field studies.

An integrated approach is crucial for SDS management, and fungicide applications play an integral role in maximizing SDS suppression (Tredway et al., 2009). Our studies demonstrated that fungicidal suppression of SDS is challenging, but it can be accomplished with certain fungicides, particularly when targeted at a specific *Ophiosphaerella* species (Tredway et al., 2020). One caveat is that many of the effective fungicides are expensive to apply across an entire facility, but site-specific targeted fungicide applications are a demonstrated way to reduce cost and optimize disease suppression (Booth et al., 2021). Moreover, optimizing fungicide applications with soil surfactants, post-application irrigation, and applying at the correct time could provide a greater

cost-benefit for the use of more expensive and effective fungicides (Hutchens et al., 2022; Walker, 2009). Our *in vitro* and *in situ* studies demonstrate which fungicides are most effective against *O. herpotricha* and *O. korrae* allowing for turfgrass managers to make more informed management decisions when targeting SDS.

References

Booth, J.C., Sullivan, D., Askew, S.A., Kochersberger, K., and McCall, D.S. 2021. Investigating targeted spring dead spot management via aerial mapping and precision-guided fungicide applications. Crop Sci. 61:3134-3144. https://doi.org/10.1002/csc2.20623

Cottrill, D.J., Earlywine, D.T., and Miller, G.L. 2016. Assessment of nitrogen source, sulfur, and fall fungicide applications on the management of spring dead spot of bermudagrass. Plant Dis. 100:473-482. doi:http://dx.doi.org/10.1094/PDIS-05-15-0565-RE

Earlywine, D.T. and Miller, G.L. 2012. Evaluation of fungicides for spring dead spot control on bermudagrass, 2010-2011. Plant Dis. Manag. Rep. 6:T022.

Earlywine, D.T. and Miller, G.L. 2013. Evaluation of fungicides for spring dead spot control on bermudagrass, 2011-2012. Plant Dis. Manag. Rep. 7:T008.

Flores, F.J., Marek, S.M., Orquera, G., and Walker, N.R. 2017. Molecular Identification and Multilocus Phylogeny of *Ophiosphaerella* Species Associated with Spring Dead Spot of Bermudagrass. Crop Sci. 57:249-261. doi:10.2135/cropsci2016.05.0437

Freund, D.R., Kerns, J.P., Butler, E.L., and Ploetz, J.N. 2019. Evaluation of fungicides for control of spring dead spot on a bermudagrass putting green, 2017-2018. Plant Dis. Manag. Rep. 13:T005.

Galle, G.H., Kerns, J.P., Butler, E.L., and Ploetz, J.N. 2019. Evaluation of Kabuto and Tekken for the control of spring dead spot on ultradwarf bermudagrass putting greens, 2017-2018. Plant Dis. Manag. Rep. 13:T006.

Hawkes, N.J. 1987 Spring dead spot of ‘Tifdwarf’ turf in south Australia. J. Sports Turf Res. Inst. J. 63:136-140.

Hutchens, W.J., Booth, J.C., Doherty, J.R., Roberts, J.A., and McCall, D.S. 2022. Influence of post-application irrigation and soil surfactants on tebuconazole efficacy against spring dead spot. Crop Pro. (in press). https://doi.org/10.1016/j.cropro.2022.105961

Hutchens, W.J., Gannon, T.W., Shew, H.D., and Kerns, J.P. 2019. Effect of post-application irrigation on fungicide movement and efficacy against *Magnaporthiopsis poae*. Crop Prot. 122:106-111. https://doi.org/10.1016/j.cropro.2019.04.027

Hutchens, W.J., Henderson, C.A., Bush, E.A., Kerns, J.P., and McCall, D.S. 2021. Geographic Distribution of *Ophiosphaerella* Species in the Mid-Atlantic United States. Plant Health Prog. https://doi.org/10.1094/PHP-04-21-0076-S

Iriarte, F.B. 2003. Genetic diversity and aggressiveness of *Ophiosphaerella korrae*, a cause of spring dead spot of bermudagrass. PhD diss. Kan. State Univ., Manhattan, KS.

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., and Tisserat, N.A. 2004. Genetic diversity and aggressiveness of *Ophiosphaerella korrae*, a cause of spring dead spot of bermudagrass. Plant Dis. 88:1341-1346.

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., Vincelli, P., Dixon, E.W., and Tisserat, N.A. 2005a. Aggressiveness of spring dead spot pathogens to bermudagrass. Int. Turf. Res. J. 10:258-264.

Iriarte, F.B., Fry, J.D., Martin, D.L., Todd, T.C., and Tisserat, N.A. 2005b. Effect of cold acclimation and freezing on spring dead spot severity in bermudagrass. Hort Sci. 40.2:421-423.

Latin, R. 2021. A Practical Guide to Turfgrass Fungicides, 2nd ed. APS Press, St. Paul, MN. p. 51-52.

Liang, H., Li, Jin., Luo, C., Li, Jia., and Fu-Xing, Z. 2019. Effects of SHAM on the Sensitivity of *Sclerotinia sclerotium* and *Botrytis cinerea* to QoI Fungicides. Plant Dis. 103:1884-1888. https://doi.org/10.1094/PDIS-12-18-2142-RE

Martin, D.L., Bell, G.E., Baird, J.H., Taliaferro, C.M., Tisserat, N.A., Kuzmic, R.M., Dobson, D.D., and Anderson, J.A. 2001. Spring Dead Spot Resistance and Quality of Seeded Bermudagrasses under Different Mowing Heights. Crop Sci. 41:451-456.

Martinez, J.F.I., Flores, F.J., Koch, A.R., Garzon, C.D., and Walker, N.R. 2019. Multiplex end-point PCR for the detection of three species of *Ophiosphaerella* causing spring dead spot of bermudagrass. Plant Dis. 103:2010-2014. https.//doi.org/10.1094/PDIS-10-18-1727-RE

Perry, D.H., Tomaso-Peterson, M., and Baird, R. 2010. Seasonal variation in frequency of isolation of *Ophiosphaerella korrae* from bermudagrass roots in Mississippi and pathogenicity and optimal growth of selected isolates. Mycologia 169:395-402. doi:10.1007/s11046-010-9273-x

Rioux, R.A., Van Ryzin, B.J., and Kerns, J.P. 2014. Development of a semi-selective medium for improved isolation of the turfgrass dollar spot pathogen *Sclerotinia homoeocarpa* from host tissues. Can. J. Plant Pathol. 36.2:235-245

Roberson, T.L., McCall, D.S., Estes, A., and Shelton, C.D. 2017. Novel Spring Dead Spot Control Using Isofetamid. Crop Science Society of America Annual Meeting, Tampa, FL.

Stephens, C.M. 2021. Etiology, epidemiology, and management of take-all root rot on golf course putting greens. Dissertation. North Carolina State Univ., Raleigh, NC. Retrieved from https://repository.lib.ncsu.edu/bitstream/handle/1840.20/38742/etd.pdf?sequence=1

Stephens, C.M., Ploetz, J.N., Butler, E.L., and Kerns, J.P. 2020. Evaluation of fungicides for the control of spring dead spot on ultradwarf bermudagrass putting greens, 2018-2019. Plant Dis. Manag. Rep. 14:T011.

Tisserat, N.A., Hulbert, S.H., and Sauer, K.M. 1994. Selective Amplification of rDNA Internal Transcribed Spacer Regions to Detect *Ophiosphaerella korrae* and *O. herpotricha*. Phytopathology 84:478-482.

Tisserat, N.A., Fry, D., Martin, D., Iriarte, F., and Wetzel III, H. 2004. Identification, distribution, and aggressiveness of spring dead spot pathogens of bermudagrass. USGA Green Sec. Rec. 42:15-18.

Tredway, L.P., Butler, E.L., Soika, M.D., and Bunting, M.L. 2008. Etiology and management of spring dead spot of hybrid bermudagrass in North Carolina, USA. Acta Hort. 783:535-546.

Tredway, L.P., Soika, M.D., Butler, E.L., and Kerns, J.P. 2020. Impact of nitrogen source, fall fertilizers, and preventative fungicides on spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha*. Crop Sci. Special Issue: International Turfgrass Research Conference: 1-10. doi:10.1002/csc2.20306

Wadsworth, D. F. and Young, H. C. 1960. Spring dead spot of bermudagrass. Plant Dis. 44:516-518.

Walker, J. and Smith, A.M. 1972. *Leptosphaeria narmari* and *L. korrae* spp. nov., two long-spored pathogens of grasses in Australia. Trans. Br. Mycol. Soc. 58.3:459-466.

Walker, N.R. 2009. Influence of Fungicide Application Timings on the Management of Bermudagrass Spring Dead Spot Caused by *Ophiosphaerella herpotricha*. Plant Dis. 93:1341-1345. https://doi.org/10.1094/PDIS-93-12-1341

Walker, N.R., Mitchell, T.K., Morton, A.N., and Marek, S.M. 2006. Influence of temperature and time of year on colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. Plant Dis. 90:1326-1330.

Wetzel, H.C., III, Skinner, D.Z., and Tisserat, N.A. 1999. Geographic distribution and genetic diversity of three *Ophiosphaerella* species that cause spring dead spot of bermudagrass. Plant Dis. 83:1160-1166.

Table 1. Effective concentrations (mg L^{-1}) of thirteen different fungicides to inhibit fungal growth by 50% (EC_{50}) of four *Ophiosphaerella herpotricha* and four *O. korrae* isolates.

		EC_{50} Values of *O. herpotricha* Isolates				**EC_{50} Values of *O. korrae* Isolates**			
Group	**Fungicide**	O. herpotricha	TC15TV-1	WH7FT-0	WH7FT-4	AAC3-2	PT001	L.korrae	AAC001
DMI[1]	fenarimol	0.35	0.23	0.44	0.60	0.17	0.33	0.27	0.16
	mefentrifluconazole	4.33	0.78	> 10	>10	0.13	0.08	0.06	0.10
	myclobutanil	0.33	0.26	0.32	0.40	0.58	0.80	0.65	0.56
	propiconazole	0.08	0.08	0.09	0.11	0.23	0.20	0.17	0.21
	tebuconazole	0.26	0.20	0.31	0.36	0.31	0.29	0.23	0.28
QoI[2]	azoxystrobin	2.78	0.68	2.36	2.68	> 10	3.98	> 10	> 10
	fluoxastrobin	2.17	0.79	1.59	1.96	> 10	> 10	> 10	> 10
	pyraclostrobin	0.06	0.05	0.06	0.08	0.30	0.06	0.10	0.27
SDHI[3]	fluopyram	0.31	0.53	0.21	0.20	0.38	0.24	0.16	0.40
	fluxapyroxad	0.09	0.17	0.05	0.05	0.32	0.15	0.19	0.33
	isofetamid	0.12	0.21	0.06	0.07	0.11	0.04	0.04	0.12
	penthiopyrad	0.04	0.07	0.03	0.02	0.19	0.06	0.08	0.19
	pydiflumetofen	0.02	0.04	0.01	0.01	0.02	0.01	0.02	0.04

[1]DMI = demethylase inhibitor
[2]QoI = quinone outside inhibitor
[3]SDHI = succinate dehydrogenase inhibitor

Table 2. Bermudagrass (*Cynodon* spp.) cultivar and predominant *Ophiosphaerella* population at locations used for fungicide field efficacy trials conducted from fall of 2019 to spring of 2021.

Location	**Bermudagrass Cultivar**	***Ophiosphaerella* Population**
Blacksburg, VA	Patriot	Inoculated with both species
Midlothian, VA	Tifway 419	Inoculated with both species
Salisbury, MD	Patriot	*O. herpotricha*
Culpeper, VA	Riviera	*O. herpotricha*
Cape Charles, VA	TifSport	*O. korrae*
Palmyra, VA	Vamont	Both species

Table 3. Fungicide group, fungicide, fungicide application rate, and number of applications for field fungicide efficacy trials conducted from fall of 2019 to spring of 2021.

Fungicide Group	Fungicide	Trade Name	Manufacturer	Rate (kg a.i. ha^{-1})	Number of Apps
Nontreated	nontreated	N/A	N/A	N/A	N/A
DMI[1]	mefentrifluconazole	Maxtima	BASF	1.02	1
DMI	mefentrifluconazole			0.51	2
DMI	myclobutanil	Eagle 20EW	Corteva	1.53	1
DMI	myclobutanil			0.76	2
DMI	propiconazole	Banner Maxx II	Syngenta	1.98	1
DMI	propiconazole			0.99	2
DMI	tebuconazole	Tebuconazole 3.6 F	Quali-Pro	1.51	1
DMI	tebuconazole			0.76	2
QoI[2]	azoxystrobin	Heritage TL	Syngenta	0.61	1
QoI	azoxystrobin			0.31	2
QoI	fluoxastrobin	Fame SC	FMC	0.55	1
QoI	fluoxastrobin			0.27	2
QoI	pyraclostrobin	Insignia SC	BASF	0.56	1
QoI	pyraclostrobin			0.28	2
SDHI[3]	fluxapyroxad	Xzemplar	BASF	0.24	1
SDHI	fluxapyroxad			0.12	2
SDHI	isofetamid	Kabuto	PBI Gordon	4.06	1
SDHI	isofetamid			2.03	2
SDHI	penthiopyrad	Velista	Syngenta	1.07	1
SDHI	penthiopyrad			0.53	2
SDHI	pydiflumetofen	Posterity	Syngenta	0.2	1
SDHI	pydiflumetofen			0.1	2

[1]DMI = demethylase inhibitor
[2]QoI = quinone outside inhibitor
[3]SDHI = succinate dehydrogenase inhibitor

Table 4. Comparison of effective concentrations (mg L^{-1}) to inhibit fungal growth by 50% (EC_{50} values) when isolates were pooled by species. Means within the same row with an asterisk are significantly different according to a Student's *t*-test (P = 0.1).

Fungicide Group	Fungicide	*O. herpotricha*	*O. korrae*
DMI[1]	fenarimol	0.41*	0.23*
	mefentrifluconazole	19.43	0.09
	myclobutanil	0.33*	0.65*
	propiconazole	0.09*	0.20*
	tebuconazole	0.28	0.28
QoI[2]	azoxystrobin	2.10	24165.60
	fluoxastrobin	1.63	1563.71
	pyraclostrobin	0.06*	0.19*
SDHI[3]	fluopyram	0.31	0.30
	fluxapyroxad	0.09*	0.25*
	isofetamid	0.11	0.08
	penthiopyrad	0.04*	0.13*
	pydiflumetofen	0.02	0.02

[1]DMI = demethylase inhibitor
[2]QoI = quinone outside inhibitor
[3]SDHI = succinate dehydrogenase inhibitor

Table 5. The influence of fungicide treatments on patch number area under the disease progress curve in 2020. Means are compared between fungicide treatments within each location. Means within the same location with the same letter are not significantly different according to a Student's *t*-test ($P < 0.1$). Fungicide effect on patch number area under the disease progress curve at locations with only dashed lines was not significant ($P < 0.1$). Blacksburg, VA and Midlothian, VA were inoculated; Salisbury, MD and Culpeper, VA were infested with *Ophiosphaerella herpotricha*; Cape Charles, VA was infested with *O. korrae*; and Palmyra, VA was infested with both *O. herpotricha* and *O. korrae*.

Fungicide	**Inoculated**		***O. herpotricha***		***O. korrae***	**Both**
	Blacksburg, VA	Midlothian, VA	Salisbury, MD	Culpeper, VA	Cape Charles, VA	Palmyra, VA
nontreated	198.0a	-	203.0a	186.6ab	341.9a	209.3a
mefentrifluconazole	43.0c	-	72.1b	67.0d	77.8bc	110.9bcd
myclobutanil	43.0c	-	47.6b	111.3a-d	215.4ab	165.0ab
propiconazole	58.0c	-	45.7b	98.8bcd	295.1a	153.3abc
tebuconazole	38.5c	-	59.4b	74.8d	264.7a	127.4bcd
azoxystrobin	61.0c	-	80.3b	175.0a	265.8a	101.8cd
fluoxastrobin	88.5bc	-	27.1b	150.6abc	277.9a	124.9bcd
pyraclostrobin	38.0c	-	163.6a	81.0cd	225.4ab	79.4de
fluxapyroxad	59.0c	-	30.6b	181.3a	255.6a	116.0bcd
isofetamid	42.5c	-	40.1b	84.0cd	11.8c	10.8f
penthiopyrad	49.0c	-	25.6b	134.4a-d	150.3abc	31.4ef
pydiflumetofen	160.0ab	-	40.1b	84.3cd	145.3abc	18.4ef

Table 6. The influence of fungicide treatments on patch number area under the disease progress curve in 2021. Means are compared between fungicide treatments within each location. Means within the same location with the same letter are not significantly different according to a Student's *t*-test ($P < 0.1$). Fungicide effect on patch number area under the disease progress curve at locations with only dashed lines was not significant ($P < 0.1$). Blacksburg, VA and Midlothian, VA were inoculated; Salisbury, MD and Culpeper, VA were infested with *Ophiosphaerella herpotricha*; Cape Charles, VA was infested with *O. korrae*; and Palmyra, VA was infested with both *O. herpotricha* and *O. korrae*.

Fungicide	**Inoculated**		***O. herpotricha***		***O. korrae***	**Both**
	Blacksburg, VA	Midlothian, VA	Salisbury, MD	Culpeper, VA	Cape Charles, VA	Palmyra, VA
nontreated	565.4a	0.0bc	274.0a	152.3abc	334.1ab	472.8a
mefentrifluconazole	174.6b-e	0.0c	59.2de	75.3c	77.4c	176.2e
myclobutanil	212.0bcd	0.0c	148.7bc	151.4abc	339.2ab	309.6bcd
propiconazole	168.2b-e	14.0ab	157.8bc	106.8bc	435.2a	186.1de
tebuconazole	128.8cde	10.3abc	164.3bc	140.9abc	360.0ab	361.1ab
azoxystrobin	261.9bc	21.5a	86.4cde	188.6ab	486.5a	178.5e
fluoxastrobin	281.1b	2.6bc	156.1bc	197.8ab	489.4a	342.6abc
pyraclostrobin	225.5bc	2.6bc	232.3ab	157.9abc	371.3a	218.5cde
fluxapyroxad	145.6b-e	7.7bc	142.9cd	241.9a	340.4ab	168.8e
isofetamid	61.7e	2.6bc	9.1e	71.3c	68.0c	26.2f
penthiopyrad	80.1de	1.2c	35.2e	135.2abc	315.1ab	30.3f
pydiflumetofen	83.5de	3.9bc	60.8de	67.8c	155.9bc	33.9f

Table 7. The influence of fungicide treatments on percent spring dead spot area under the disease progress curve in 2020. Means are compared between fungicide treatments within each location. Means within the same location with the same letter are not significantly different according to a Student's *t*-test ($P < 0.1$). Fungicide effect on percent spring dead spot area under the disease progress curve at locations with only dashed lines was not significant ($P < 0.1$). Blacksburg, VA and Midlothian, VA were inoculated; Salisbury, MD and Culpeper, VA were infested with *Ophiosphaerella herpotricha*; Cape Charles, VA was infested with *O. korrae*; and Palmyra, VA was infested with both *O. herpotricha* and *O. korrae*.

Fungicide	**Inoculated**		***O. herpotricha***		***O. korrae***	**Both**
	Blacksburg, VA	Midlothian, VA	Salisbury, MD	Culpeper, VA	Cape Charles, VA	Palmyra, VA
nontreated	-	-	109.1ab	141.9abc	-	177.1a
mefentrifluconazole	-	-	97.0ab	54.9d	-	65.3c
myclobutanil	-	-	51.9b	99.7a-d	-	127.2ab
propiconazole	-	-	48.8b	65.3cd	-	131.6ab
tebuconazole	-	-	101.3ab	68.8bcd	-	66.5c
azoxystrobin	-	-	65.5b	145.5a	-	60.9cd
fluoxastrobin	-	-	25.2b	135.2ab	-	87.9bc
pyraclostrobin	-	-	160.8a	70.6bcd	-	61.0c
fluxapyroxad	-	-	13.2b	150.0a	-	87.0bc
isofetamid	-	-	57.6b	87.9a-d	-	8.9e
penthiopyrad	-	-	22.8b	109.9a-d	-	14.5de
pydiflumetofen	-	-	40.1b	86.9a-d	-	12.1e

Table 8. The influence of fungicide treatments on percent spring dead spot area under the disease progress curve in 2021. Means are compared between fungicide treatments within each location. Means within the same location with the same letter are not significantly different according to a Student's *t*-test (P < 0.1). Fungicide effect on percent spring dead spot area under the disease progress curve at locations with only dashed lines was not significant (P < 0.1). Blacksburg, VA and Midlothian, VA were inoculated; Salisbury, MD and Culpeper, VA were infested with *Ophiosphaerella herpotricha*; Cape Charles, VA was infested with *O. korrae*; and Palmyra, VA was infested with both *O. herpotricha* and *O. korrae*.

Fungicide	**Inoculated**		***O. herpotricha***		***O. korrae***	**Both**
	Blacksburg, VA	Midlothian, VA	Salisbury, MD	Culpeper, VA	Cape Charles, VA	Palmyra, VA
nontreated	389.0a	0.0bc	186.8abc	-	368.8a-d	365.1a
mefentrifluconazole	99.0bcd	0.0c	94.4b-f	-	90.8d	131.9cde
myclobutanil	152.0b	0.0c	153.3abc	-	432.4abc	242.3abc
propiconazole	102.8bcd	9.1abc	146.3a-d	-	477.5ab	192.2bcd
tebuconazole	80.4bcd	6.2abc	189.2ab	-	379.6bc	294.3ab
azoxystrobin	107.1bcd	14.1a	78.2c-f	-	425.8abc	140.1cd
fluoxastrobin	120.2bc	0.4c	124.6b-e	-	659.1a	221.1bcd
pyraclostrobin	121.5bc	0.6c	229.8a	-	403.1abc	160.9cd
fluxapyroxad	64.2bcd	11.6ab	113.1b-e	-	438.7abc	124.3de
isofetamid	19.9d	0.5c	5.7f	-	90.4d	23.4e
penthiopyrad	39.4cd	0.4c	22.1ef	-	328.9bcd	26.8e
pydiflumetofen	36.6cd	1.4c	44.0def	-	191.4cd	25.8e

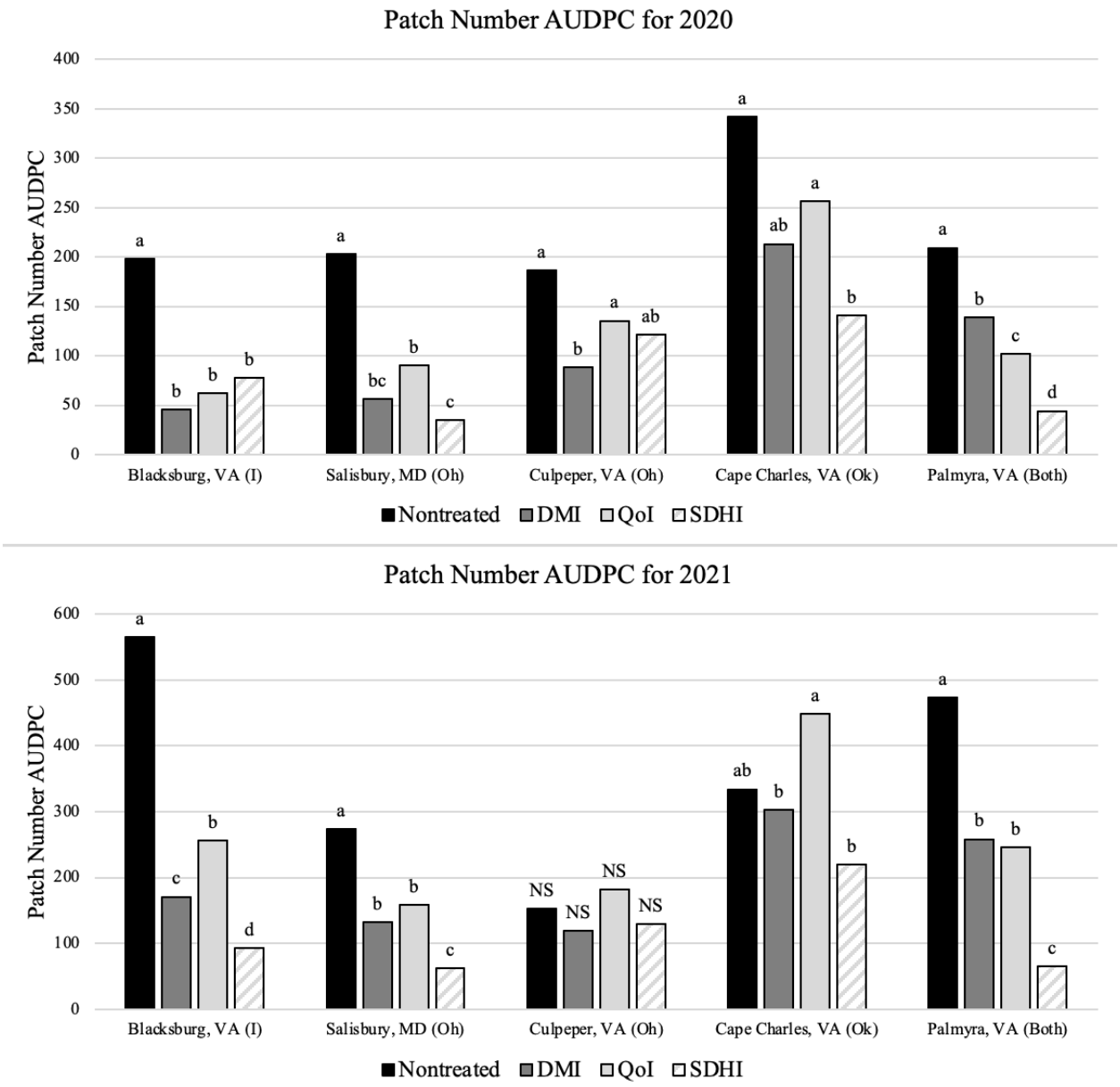

Figure 1. The influence of fungicide group (DMI = demethylase inhibitor; QoI = quinone outside inhibitor; SDHI = succinate dehydrogenase inhibitor) on patch number area under the disease progress curve for 2020 and 2021. Means are compared between fungicide groups within each location. Means within the same location with the same letter are not significantly different according to a Student's *t*-test ($P < 0.1$). The characters "NS" represent non-significance. The study was conducted at Blacksburg, VA which was inoculated with both *O. herpotricha* and *O. korrae*; Salisbury, MD and Culpeper, VA were infested with *Ophiosphaerella herpotricha*; Cape Charles, VA was infested with *O. korrae*; and Palmyra, VA was infested with both *O. herpotricha* and *O. korrae*.

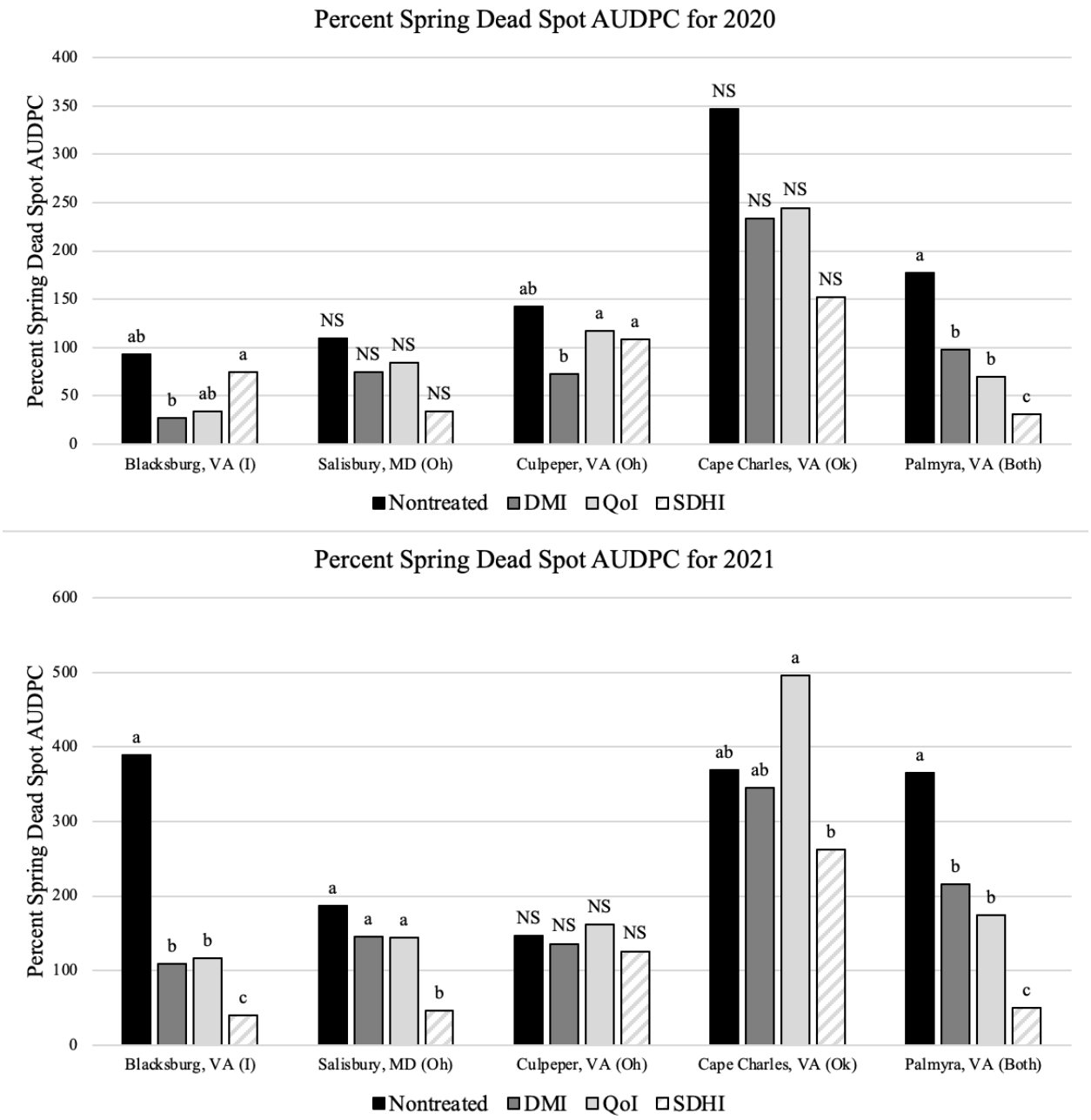

Figure 2. The influence of fungicide group (DMI = demethylase inhibitor; QoI = quinone outside inhibitor; SDHI = succinate dehydrogenase inhibitor) on percent spring dead spot area under the disease progress curve for 2020 and 2021. Means are compared between fungicide groups within each location. Means within the same location with the same letter are not significantly different according to a Student's *t*-test ($P < 0.1$). The characters "NS" represent non-significance. The study was conducted at Blacksburg, VA which was inoculated with both *O. herpotricha* and *O. korrae*; Salisbury, MD and Culpeper, VA were infested with *Ophiosphaerella herpotricha*; Cape Charles, VA was infested with *O. korrae*; and Palmyra, VA was infested with both *O. herpotricha* and *O. korrae*.

Chapter 4: Influence of Post-Application Irrigation and Soil Surfactant on Tebuconazole Efficacy against Spring Dead Spot

Abstract

Spring dead spot (SDS) (*Ophiosphaerella* spp.) causes damage to hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) grown in areas where winter dormancy occurs. The pathogen infects the stolons, rhizomes, and roots of warm-season grasses. Symptoms appear as circular, necrotic patches at spring greenup that reduce the playability and aesthetics of bermudagrass. Historically, fungicide efficacy against SDS has been inconsistent. There may be opportunities to improve application and post-application practices to mitigate the inconsistency. A study was conducted from 2019 to 2021 to examine the influence of post-application irrigation and soil surfactant on tebuconazole efficacy against SDS. The study was conducted at three locations: Virginia Tech Turfgrass Research Center (TRC), Blacksburg, VA; Independence Golf Club (IGC), Midlothian, VA; Nutters Crossing Golf Club (NCGC), Salisbury, MD. Tebuconazole was applied in the fall either once at 1.5 kg ai ha^{-1} or twice at 1.5 kg ai ha^{-1} two to four weeks apart when soil temperatures were between 10.7 and 21.8°C. Treatments were applied with or without a soil surfactant and with or without 0.6 cm of post-application irrigation. Bermudagrass was assessed the following spring two or three times for patch number and percent SDS. Data were analyzed by assessment date, subjected to analysis of variance, and means were separated using Tukey's Honest Significant Difference test (P = 0.05). There were no treatment differences at IGC or NCGC in 2020 or 2021. At the TRC in both 2020 and 2021, results were inconsistent with tebuconazole generally suppressing SDS compared to the nontreated control. However, differences between tebuconazole-treated plots were variable. Our study suggests that including a

soil surfactant with tebuconazole applications and/or irrigating post-application does not consistently increase SDS suppression.

Introduction

Spring dead spot (SDS), caused by multiple *Ophiosphaerella* spp., is a detrimental disease of hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) grown in areas where winter dormancy is induced by cold temperatures (Wadsworth and Young, 1960; Wetzel et al., 1999). The pathogens infect the rhizomes, stolons, and roots primarily in the fall, but are active when soil temperatures range from 8 to 30°C (Perry et al., 2010; Tredway et al., 2009; Walker et al., 2006). As spring green-up progresses, symptoms are expressed as isolated, necrotic patches that range from a few centimeters to > 1 meter in diameter (Wadsworth and Young, 1960). Spring dead spot causes turf loss, reduced playability, and increased weed pressure (Martin et al., 2001; McCarty et al., 1991). There are three species that cause SDS: *O. herpotricha* (Fr:Fr) J. Walker, *O. korrae* (J. Walker & A.M. Smith) Shoemaker & C.E. Babcock (=*Leptosphaeria korrae* J. Walker & A.M. Smith), and *O. narmari* (J. Walker & A.M. Smith) Wetzel, Hulbert, & Tisserat (=*L. narmari* J. Walker & A.M. Smith). These species respond differently to many cultural and chemical management practices (Cottrill et al., 2016; Hutchens et al., 2019b; Tredway et al., 2020).

Certain cultural practices effectively reduce SDS severity. Miller et al. (2017) found that aggressive cultivation practices such as fraze mowing in the summer could reduce SDS severity the following spring. Similarly, Tisserat and Fry (1997) observed that verticutting and aerification in the summer reduced SDS symptoms the following spring. Aggressive cultivation in the summer is a beneficial practice for SDS prevention likely because it reduces thatch and generates new growing points on the stems (Miller et al., 2017). Thatch reduction can create a less conducive environment for many plant pathogens and reducing thatch can also help fungicides reach the roots (Allan-Perkins et al., 2018; Dell et al., 1994). Fertility practices can also be beneficial for preventing and recovering from SDS damage, but results have been inconsistent (Cottrill et al.,

2016; Dernoeden et al., 1991; Hutchens et al., 2021a; McCarty et al., 1992; Tredway et al., 2020). Tredway et al. (2020) determined that calcium nitrate and ammonium sulfate were able to effectively suppress *O. korrae* and *O. herpotricha*, respectively. Cottrill et al. (2016) found that sulfur was able to reduce SDS severity in the field, while nitrogen source had no effect. In contrast, McCarty et al. (1992) determined that sulfur-coated urea and potassium sulfate increased SDS damage. While there are discrepancies on the effect of fertility on SDS prevention, fertilizer applications in the spring and early summer have consistently been shown to increase bermudagrass recovery from damage (Dernoeden et al., 1991; Hutchens et al., 2021a). Although certain cultural practices can mitigate SDS, many of them do not provide economically acceptable suppression of the disease, so fungicide applications are necessary.

Fungicides are commonly applied to prevent SDS but, similar to fertilizer applications, results have been inconsistent (Earlywine and Miller, 2012; Earlywine and Miller, 2013; Freund et al., 2019; Kerns et al., 2017; Stephens et al., 2020). Tebuconazole followed by tebuconazole + iprodione applications suppressed SDS compared to a nontreated control in 2012, but the same combination did not suppress SDS the previous year (Earlywine and Miller, 2012; Earlywine and Miller, 2013). Penthiopyrad reduced SDS area under the disease progress curve compared to a nontreated control on an ultradwarf bermudagrass putting green in 2016, but did not in 2019 (Kerns et al., 2017; Stephens et al., 2020). Freund et al. (2019) also found that penthiopyrad reduced SDS patch area compared to a nontreated control. Fenarimol did not reduce SDS severity in an Earlywine and Miller (2013) study, but it reduced SDS incidence in a Tredway et al. (2007) study. Fungicide efficacy is variable based on geographic region, timing of the application, *Ophiosphaerella* population, and fungicide application method (Butler and Tredway, 2006; Hutchens et al., 2019b; Tredway et al., 2020; Walker, 2006). Fungicide efficacy could also be

influenced by thatch or soil organic matter as many fungicides are readily bound by organic carbon (Hutchens et al., 2019a). Methods to optimize fungicide applications for SDS suppression have not been widely explored and have provided mixed results (Beck et al., 2012; Butler et al., 2006; Earlywine and Miller, 2015; Tredway et al., 2008; Walker, 2013).

Post-application irrigation and soil surfactants are commonly applied to increase fungicide efficacy against crown- and root-infecting pathogens by increasing downward movement of the fungicide to the basal portions of the plant where the pathogens infect (Beck et al., 2012; Gannon et al., 2017; Hutchens et al., 2019a; Hutchens et al., 2020a; Stephens et al., 2021). Gannon et al. (2017) showed increase in downward distribution of the nematicide abamectin with both post-application irrigation and soil surfactant applications. Hutchens et al. (2020a) showed that soil surfactants increase downward movement of azoxystrobin, myclobutanil, and propiconazole in a bare 90:10 (90% sand: 10% peat) United States Golf Association putting green soil. Moreover, Hutchens et al. (2019a) exhibited an increase in azoxystrobin downward movement in the soil and an increased efficacy against the crown- and root-infecting pathogen *Magnaporthiopsis poae*, the cause of summer patch of creeping bentgrass, when post-application irrigation was applied.

Although post-application irrigation and soil surfactant applications have increased fungicide performance against many root-infecting pathogens, the effect of post-application irrigation and soil surfactants on fungicide efficacy against SDS has been inconsistent (Beck et al., 2012; Butler and Tredway, 2006; Earlywine and Miller, 2015; Kerns et al., 2017; Tredway et al., 2008; Walker, 2013). Post-application irrigation did not increase penthiopyrad efficacy against SDS in a Walker (2013) study. Disease pressure was low during this study which may have diluted treatment effects. Kerns et al. (2017) observed penthiopyrad and penthiopyrad + azoxystrobin + difenoconazole applications suppressing SDS similarly, regardless of post-application irrigation

amount. Moreover, fungicide application methods have shown to have an effect on SDS incidence in certain years and locations while not having an effect in others (Tredway et al., 2008). Soil surfactants tank-mixed with tebuconazole did not increase efficacy against SDS compared to tebuconazole alone (Earlywine and Miller, 2015). In contrast, Beck et al. (2012) showed increased efficacy of fenarimol and fenarimol + thiophanate-methyl against SDS when applied in conjunction with a soil surfactant. The inconsistencies in fungicide efficacy against SDS and the limited research available on fungicide application methods for SDS warrant further investigation into methods to optimize fungicide applications for SDS suppression. Therefore, the objective of this field study was to assess the impact of post-application irrigation and a soil surfactant on suppression of SDS with tebuconazole—a commonly applied fungicide for SDS prevention.

Materials and Methods

Site description

A study examining the effect of post-application irrigation and soil surfactant on tebuconazole efficacy against SDS was conducted from the fall of 2019 to the spring of 2020 and repeated using the same plots for each treatment in the fall of 2020 to the spring of 2021. The study was conducted at three different locations: Independence Golf Club (IGC), Midlothian, VA; Nutters Crossing Golf Club (NCGC), Salisbury, MD; and Virginia Tech Turfgrass Research Center (TRC), Blacksburg, VA. The TRC and NCGC locations were 'Patriot' hybrid bermudagrass fairways mown at 15 mm two to three times per week during active growing periods. The study at IGC was conducted on a 'Tufcote' hybrid bermudagrass tee box mown at 13 mm three times per week during active growing periods. Fertilizer, herbicides, and irrigation were applied as needed to maintain a healthy, uniform turf. All locations had natural infestations of SDS with each location having a majority *O. herpotricha* population as reported in previous research

(Hutchens et al., 2021b). The IGC and TRC locations were also inoculated with both *O. herpotricha* and *O. korrae*, however, there were no obvious symptoms in the inoculation points for the duration of the trial, so the authors assume that the observed SDS symptoms were likely from a natural infestation of *O. herpotricha*.

Study design and treatments

The study was a randomized complete block design (RCBD) with nine treatments: 1) nontreated control, 2) one application of tebuconazole (1.5 kg ai ha^{-1}) [Tebuconazole 3.6 F, Quali-Pro, Houston, TX], 3) one application of tebuconazole (1.5 kg ai ha^{-1}) + one application of soil surfactant (50.9 L ha^{-1}) [Hydra-Last, Landscape Supply Inc., Roanoke, VA], 4) one application of tebuconazole (1.5 kg ai ha^{-1}) + 0.6 cm of post-application irrigation, 5) one application of tebuconazole (1.5 kg ai ha^{-1}) + one application of soil surfactant (50.9 L ha^{-1}) + 0.6 cm of post-application irrigation, 6) two applications of tebuconazole (1.5 kg ai ha^{-1} per application), 7) two applications of tebuconazole (1.5 kg ai ha^{-1} per application) + two applications of soil surfactant (25.5 L ha^{-1} per application), 8) two applications of tebuconazole (1.5 kg ai ha^{-1} per application) + 0.6 cm of post-application irrigation, 9) two applications of tebuconazole (1.5 kg ai ha^{-1} per application) + two applications of soil surfactant (25.5 L ha^{-1} per application) + 0.6 cm of post-application irrigation. All treatments were applied with TTI 11004-VP nozzles [TeeJet Technologies, Glendale Heights, IL] to 1.8 x 2.7 m plots with a CO_2-pressurized sprayer delivering solution at 276 kPa of pressure with a water carrier volume of 842 L ha^{-1}. Treatments that received post-application irrigation were irrigated within 30 min with 0.6 cm of water. Irrigation was applied by hand-watering to individual plots, and the length of time to irrigate each plot to deliver 0.6 cm of water was calibrated for each site prior to treatment. Treatments that did not receive post-application irrigation were applied within 1 hr after irrigation when surface water had drained.

Applications were made in the fall when five-day average soil temperatures at 0 to 10 cm were between 10.7 and 21.8°C (Table 1). Five-day average soil temperatures were estimated using the Syngenta GreenCast Application [https://www.greencastonline.com/tools/soil-temperature; Syngenta Crop Protection, Greensboro, NC].

Data collection and analysis

Plots at IGC and TRC were visually assessed on three separate dates the following spring and early summer after fall treatments for patch count and percent SDS. Plots at NCGC were assessed on three separate dates in the spring and early summer of 2020 and two separate dates in the spring of 2021 for percent SDS. Patch count data was collected at NCGC on two assessment dates in 2020, yet it was not collected in the spring of 2021. Percent SDS and patch count data were subjected to analysis of variance (ANOVA) and means were separated when appropriate using a Tukey's Honest Significant Difference test ($P < 0.05$) in JMP Pro 15 [SAS Institute, Cary, NC].

Results

Independence Golf Club

Disease pressure varied across sites with IGC having low disease pressure in both 2020 and 2021. The nontreated control had ≤ 1.5% SDS on the assessment date with the greatest amount of disease in 2020 and ≤ 8.33% SDS on the assessment date with the greatest amount of disease in 2021 (Table 2). There were no treatment differences in patch number or % SDS at IGC in 2020 or 2021 (Table 2).

Nutters Crossing Golf Club

Disease pressure was low at NCGC in 2020 with the nontreated control having ≤ 7.5% SDS on the assessment date with the greatest amount of disease (Table 3). However, disease

pressure was higher in 2021 with the nontreated control having up to 23.75% SDS on 5 May 2021. Like IGC, there were no treatment differences in patch number or % SDS at NCGC in 2020 or 2021 ($P \geq 0.0613$).

Virginia Tech Turfgrass Research Center

Disease pressure was low at the TRC in 2020 with the nontreated control having ≤ 4.88% SDS on the assessment date with the greatest amount of disease (Table 4). On 22 May 2020, all tebuconazole-treated plots had ≥ 55% lower patch numbers and ≥ 65% less % SDS than the nontreated control. On 05 Jun 2020, only the treatments with two applications of tebuconazole with a soil surfactant reduced patch number (≥ 95%) compared to the nontreated control. There were no treatment differences at the TRC on 22 Jun 2020 likely from the bermudagrass mostly recovering from the SDS damage.

In 2021, the TRC had higher disease pressure than 2020 with the nontreated control having up to 12.83% SDS on 15 May 2021 (Table 4). On 15 May 2021, all treatments except for one application of tebuconazole without post-application irrigation or soil surfactant reduced patch number compared to the nontreated control. However, on the same assessment date, one application of tebuconazole, one application of tebuconazole + post-application irrigation, and two applications of tebuconazole + two applications of soil surfactant had the same % SDS as the nontreated control. On 03 Jun 2021, one application of tebuconazole and one application of tebuconazole + post-application irrigation did not reduce patch number compared to the nontreated control—all other treatments did by at least 63%. On that same assessment date, only one application of tebuconazole + one application of soil surfactant + post-application irrigation, two applications of tebuconazole, two applications of tebuconazole + post-application irrigation, and two applications of tebuconazole + two applications of soil surfactant + post-application irrigation

reduced % SDS (≥ 80%) compared to the nontreated control, yet no tebuconazole treatments were different. All tebuconazole treatments reduced patch number on 22 Jun 2021 by ≥ 58% compared to the nontreated control, and no tebuconazole treatments were different from each other. One application of tebuconazole + one application of soil surfactant, two applications of tebuconazole, two applications of tebuconazole + post-application irrigation, and two applications of tebuconazole + two applications of soil surfactant + post-application irrigation were the only treatments to reduce % SDS (≥ 80%) compared to the nontreated control on 22 Jun 2021. All tebuconazole treatments except one application of tebuconazole without post-application irrigation or soil surfactant reduced patch number on multiple assessments compared to the nontreated control. Those plots receiving one application of tebuconazole without post-application irrigation or soil surfactant reduced patch numbers on 22 Jun 2021 but not on other assessment dates.

Discussion

Previous research on soil surfactants shows that they can increase fungicide distribution in soil, which could potentially translate to increased efficacy against crown- and root-infecting pathogens such as *Ophiosphaerella* spp. (Hutchens et al., 2020a). On 05 Jun 2020, at the TRC location, two applications of tebuconazole with soil surfactant with or without post-application irrigation were the only treatments to reduce SDS patch number. Beck et al. (2012) also observed increased fungicide efficacy against SDS on a bermudagrass fairway with the inclusion of soil surfactant. These data highlight the potential for soil surfactants to increase fungicide efficacy against crown- and root-infecting pathogens such as *Ophiosphaerella* spp., yet care must be taken when applying soil surfactants in the fall as phytotoxicity was observed at NCGC on 16 Nov 2019, particularly in plots that received a soil surfactant application without post-application irrigation

(data not shown). Phytotoxic effects of soil surfactants have been previously reported, particularly if the soil surfactants did not receive post-application irrigation (Karnok, 2006).

Our data showed that post-application irrigation did not consistently increase tebuconazole efficacy against SDS. However, post-application irrigation can increase fungicide distribution in soil as well as increase fungicide efficacy against the disease summer patch caused by the crown- and root-infecting pathogen *M. poae* (Hutchens et al., 2019a; Stephens et al., 2021). There was one example in our study that suggested not applying a soil surfactant or post-application irrigation could be detrimental to tebuconazole efficacy against SDS. Applying tebuconazole only once without a soil surfactant or post-application irrigation proved to be detrimental on 15 May 2021 at the TRC. On this assessment date, tebuconazole applied once without a soil surfactant or post-application irrigation did not reduce SDS patch number compared to the nontreated control—the only treatment not to on 15 May 2021. This suggests that not applying a soil surfactant and/or post-application irrigation could be detrimental for turfgrass managers applying fungicides targeted at crown- and root-infecting pathogens. But, generally, in our study, post-application irrigation and soil surfactant did not consistently increase tebuconazole efficacy against SDS.

Our data suggest that tebuconazole efficacy was inconsistent across years and locations, which is similar to what others have found (Earlywine and Miller, 2012; Earlywine and Miller, 2013; Freund et al., 2019; Kerns et al., 2017; Stephens et al., 2020). However, we did observe, when comparing one application of tebuconazole in the fall to two applications, that two applications of tebuconazole can lead to greater SDS suppression than one application at certain locations on certain assessment dates (data not shown). We did not observe any differences when comparing the tebuconazole treatments that included a soil surfactant to tebuconazole treatments that did not include a soil surfactant or when comparing tebuconazole treatments that received

post-application irrigation to tebuconazole treatments that did not receive post-application irrigation (data not shown). This suggests that increasing the number of tebuconazole applications in the fall could be more beneficial than irrigating the product in or including a soil surfactant with the application. Tebuconazole is moderately efficacious against *O. herpotricha* and *O. korrae in vitro*, which suggests it would be effective in the field (Hutchens et al., 2019b). However, tebuconazole has a high K_{oc} value (470-6000 ml g^{-1}) making it difficult, regardless of post-application irrigation or soil surfactant application, to move the fungicide to the underground portions of the turfgrass plant where *O. herpotricha* and *O. korrae* infect (Hutchens et al., 2019a; National Center for Biotechnology Information, 2022). This suggests that either more mobile and/or more efficacious fungicides are required to maximize SDS suppression. Most fungicides are not highly mobile, so highly efficacious fungicides should be targeted to allow for maximum SDS suppression. Many of the highly efficacious fungicides such as isofetamid, mefentrifluconazole, penthiopyrad, and pydiflumetofen are more costly than tebuconazole, but the advent of GPS-guided sprayers and site-specific fungicide applications for SDS have reduced the cost of fungicide applications by up to 65% making the more expensive, efficacious products economically feasible (Booth et al., 2021; Freund et al., 2019; Kerns et al., 2017; Hutchens et al., 2019b, 2020b; Stephens et al., 2020).

Our data suggest that the application methods we tested did not consistently improve tebuconazole efficacy against SDS. Tebuconazole is a widely applied fungicide for SDS suppression, yet the reasons for its inconsistent efficacy against SDS are still not fully understood. The advent of new, more efficacious fungicides makes SDS suppression more manageable. Future research should be conducted to determine the influence of soil surfactant and post-application

irrigation on the efficacy of more potent chemistries with lower K_{oc} values against SDS to optimize fungicidal suppression of the disease.

Acknowledgements

The authors would like to acknowledge the financial support of the Golf Course Superintendent Association of America's Environmental Institute for Golf, the Mid-Atlantic Association of Golf Course Superintendents, the Eastern Shore Association of Golf Course Superintendents, the Mid-Atlantic Sports Turf Managers Association, the Old Dominion Golf Course Superintendents Association, the Virginia Golf Course Superintendents Association, and the Virginia Turfgrass Foundation on this project. The authors would also like to thank Pat Riebe, golf course superintendent at Nutters Crossing Golf Course; Dan Taylor, golf course superintendent at Independence Golf Club; and Jon Dickerson, turf farm manager at Virginia Tech Turfgrass Research Center, for their help with this project and allowing us to conduct research at their facilities.

References

Allan-Perkins, E., Manter, D., and Jung, G. 2018. Abundance of Bacteria, Fungi, and *Sclerotinia homoeocarpa* in the Thatch and Soil of Golf Courses. Phytobiomes J. 2:71-81. https://doi.org/10.1094/PBIOMES-09-17-0036-R

Beck, L.L., Moore-Kucera, J., Henry, G., Woodward, J., Zak, J., and Cox, R. 2012. Evaluation of Chemical and Cultural Methods for the Management of Spring Dead Spot in Bermudagrass Turf. Dissertation. Texas Tech Univ., Lubbock, TX. Retrieved from https://ttu-ir.tdl.org/bitstream/handle/2346/50750/Beck_Leslie_Diss.pdf?sequence=1&isAllowed=y

Booth, J.C., McCall, D.S., Sullivan, D., Askew, S.A., and Kochersberger, K. 2021. Investigating Targeted Spring Dead Spot Management via Aerial Mapping and Precision-Guided Fungicide Applications. Crop Sci. Special Issue: International Turfgrass Research Conference. https://doi.org/10.1002/csc2.20623.

Butler, E.L. and Tredway, L.P. 2006. Method and timing of fungicide applications for control of spring dead spot in hybrid bermudagrass. Online. Plant Health Prog. https://doi.org/10.1094/PHP-2006-0901-01-RS

Cottrill, D.J., Earlywine, D.T., and Miller, G.L. 2016. Assessment of nitrogen source, sulfur, and fall fungicide applications on the management of spring dead spot of bermudagrass. Plant Dis. 100:473-482. https://doi.org/10.1094/PDIS-05-15-0565-RE

Dell, C.J., Throssell, C.S., Bischoff, M., and Turco, R.F. 1994. Estimation of Sorption Coefficients for Fungicides in Soil and Turfgrass Thatch. J. Environ. Qual. 23:92-96. https://doi.org/10.2134/jeq1994.00472425002300010013x

Dernoeden, P.H., Crahay, J.N., and Davis, D.B. 1991. Spring dead spot and bermudagrass quality as influenced by nitrogen source and potassium. Crop Sci. 31:1674-1680. https://doi.org/10.2135/cropsci1991.0011183X003100060058x

Earlywine, D.T. and Miller, G.L. 2012. Evaluation of fungicides for spring dead spot control on bermudagrass, 2010-2011. Plant Dis. Manag. Rep. 6:T022.

Earlywine, D.T. and Miller, G.L. 2013. Evaluation of fungicides for spring dead spot control on bermudagrass, 2011-2012. Plant Dis. Manag. Rep. 7:T008.

Earlywine, D.T. and Miller, G.L. 2015. Evaluation of multiple fungicides in combination with a wetting agent for spring dead spot control on bermudagrass, 2013-2014. Plant Dis. Manag. Rep. 9:T018.

Freund, D.R., Kerns, J.P., Butler, E.L., and Ploetz, J.N. 2019. Evaluation of fungicides for control of spring dead spot on a bermudagrass putting green, 2017-2018. Plant Dis. Manag. Rep. 13:T005.

Gannon, T.W., Jeffries, M.D., and Ahmed, K.A. 2017. Irrigation and Soil Surfactants Affect Abamectin Distribution in Soil. Crop Sci. 57.2:573-580. https://doi.org/10.2135/cropsci2016.05.0320

Hutchens, W.J., Gannon, T.W., Shew, H.D., and Kerns, J.P. 2019a. Effect of post-application irrigation on fungicide movement and efficacy against *Magnaporthiopsis poae*. Crop Prot. 122:106-111. https://doi.org/10.1016/j.cropro.2019.04.027

Hutchens, W.J., Nagaoka, Y., Kerns, J. P., Goatley, J. M., Nita, M., & McCall, D. S. 2019b. Variable sensitivity of *Ophiosphaerella* spp. causing spring dead spot to fungicides and temperature. Paper 120074. Paper presented at the annual meeting of ASA, CSSA, and SSSA, San Antonio, TX.

Hutchens, W.J., Gannon, T.W., Shew, H.D., Ahmed, K.A., and Kerns, J.P. 2020a. Soil surfactants influence fungicide movement in United States Golf Association putting green soil. J. Environ. Qual. 49:450-459. https://doi.org/10.1002/jeq2.20021

Hutchens, W.J., Nagaoka, Y., Kerns, J.P., Goatley, J.M., Nita, M., Booth, J.C., and McCall, D.S. 2020b. Differential Response of *Ophiosphaerella* Species *In Situ* to Various Fungicides. Crop Science Society of America Annual Meeting (Virtual).

Hutchens, W.J., Booth, J.C., Hensler, K.L., Goatley, J.M., and McCall, D.S. 2021a. Cultural and fertility practices influence hybrid bermudagrass recovery from spring dead spot damage (in review).

Hutchens, W.J., Henderson, C.A., Bush, E.A., Kerns, J.P., and McCall, D.S. 2021b. Geographic Distribution of *Ophiosphaerella* Species in the Mid-Atlantic United States. Plant Health Prog. https://doi.org/10.1094/PHP-04-21-0076-S

Karnok, K.J. 2006. Which wetting agent is best? Golf Course Manage. 74:82-83.

Kerns, J.P., Butler, E.L., Soika, M.D., and Ploetz, J.N. 2017. Effects of Velista and Briskway programs on control of spring dead spot on a bermudagrass putting green, 2015-2016. Plant Dis. Manag. Rep. 11:T020.

Martin, D.L., Bell, G.E., Baird, J.H., Taliaferro, C.M., Tisserat, N.A., Kuzmic, R.M., Dobson, D.D., and Anderson, J.A. 2001. Spring Dead Spot Resistance and Quality of Seeded Bermudagrasses under Different Mowing Heights. Crop Sci. 41:451-456. https://doi.org/10.2135/cropsci2001.412451x

McCarty, L.B., DiPaola, J.M., and Lucas, L.T. 1991. Regrowth of Bermudagrass Infected with Spring Dead Spot Following Low Temperature Exposure. Crop Sci. 31:182-184. https://doi.org/10.2135/cropsci1991.0011183X003100010041x

McCarty, L.B., Lucas, L.T., and DiPaola, J.M. 1992. Spring Dead Spot Occurrence in Bermudagrass following Fungicide and Nutrient Applications. HortSci. 27.10:1092-1093. https://doi.org/10.21273/HORTSCI.27.10.1092

Miller, G.L., Earlywine, D.T., and Fresenburg, B.S. Effect of Fraze Mowing on Spring Dead Spot Caused by *Ophiosphaerella herpotricha* of Bermudagrass. 2017. Int. Turfgrass Soc. Res. J. 13:225-228. https://doi.org/10.2134/itsrj2016.10.0839

National Center for Biotechnology Information. PubChem Compound Database CID=86102. https://pubchem.ncbi.nlm.nih.gov/compound/Tebuconazole#section=Hazards-Identification, Accessed 31 Jan 2022

Perry, D.H., Tomaso-Peterson, M., and Baird, R. 2010. Seasonal variation in frequency of isolation of *Ophiosphaerella korrae* from bermudagrass roots in Mississippi and pathogenicity and optimal growth of selected isolates. Mycopathologia 169:395-402. https://doi.org/10.1007/s11046-010-9273-x

Stephens, C.M., Ploetz, J.N., Butler, E.L., and Kerns, J.P. 2020. Evaluation of fungicides for the control of spring dead spot on ultradwarf bermudagrass putting greens, 2018-2019. Plant Dis. Manag. Rep. 14:T011.

Stephens, C.M., Kerns, J.P., Ahmed, K.A., and Gannon, T.W. 2021. Influence of post-application irrigation and mowing timing on fungicide fate on a United States Golf Association golf course putting green. J. Environ. Qual. 50:868-876. https://doi.org/1002/jeq2.20249

Tisserat, N.A. and Fry, J.D. 1997. Cultural practices to reduce spring dead spot (*Ophiosphaerella herpotricha*) severity in *Cynodon dactylon*. J. Intl. Turf. Res. 8:931-936.

Tredway, L.P., Butler, E.L., and Soika, M.D. 2007. Evaluation of fungicides for preventative control of spring dead spot in bermudagrass, 2005-2006. Plant Dis. Manag. Rep. 1:T030.

Tredway, L.P., Soika, M.D., Butler, E.L., and Kerns, J.P. 2020. Impact of nitrogen source, fall fertilizers, and preventative fungicides on spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha*. Crop Sci. Special Issue: International Turfgrass Research Conference: 1-10. https://doi.org/10.1002/csc2.20306

Tredway, L. P., Tomaso-Peterson, M., Perry, H., and Walker, N. R. 2009. Spring dead spot of bermudagrass: A challenge for researchers and turfgrass managers. Online. Plant Health Progress doi:10.1094/PHP-2009-0710-01-RV.

Wadsworth, D. F. and Young, H. C. 1960. Spring dead spot of bermudagrass. Plant Dis. 44:516-518.

Walker, N.R. 2006. Evaluation of timing and application frequency of tebuconazole for the management of spring dead spot of bermudagrass, 2004-2005. Plant Dis. Manag. Rep. 1:T051.

Walker, N.R. 2013. Evaluation of post fungicide application irrigation on control of spring dead spot of bermudagrass in Oklahoma, 2012-2013. Plant Dis. Manag. Rep. 8:T011.

Walker, N.R., Mitchell, T.K., Morton, A.N., and Marek, S.M. 2006. Influence of temperature and time of year on colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. Plant Dis. 90:1326-1330. https://doi.org/10.1094/PD-90-1326

Wetzel, H.C., III, Skinner, D.Z., and Tisserat, N.A. 1999. Geographic distribution and genetic diversity of three *Ophiosphaerella* species that cause spring dead spot of bermudagrass. Plant Dis. 83:1160-1166. https://doi.org/10.1094/PDIS.1999.83.12.1160

Table 1. Date and five-day average soil temperature data for fungicide and soil surfactant applications made in the fall of 2019 and 2020 at the Virginia Tech Turfgrass Research Center (TRC), Blacksburg, VA; Independence Golf Club (IGC), Midlothian, VA; and Nutters Crossing Golf Club (NCGC), Salisbury, MD.

	Location											
	TRC				**IGC**				**NCGC**			
Year	2019		2020		2019		2020		2019		2020	
Application number	1	2	1	2	1	2	1	2	1	2	1	2
Application date	19 Sep	16 Oct	16 Sep	8 Oct	10 Oct	7 Nov	23 Sep	14 Oct	17 Oct	6 Nov	24 Sep	14 Oct
5-day avg. soil temp. (°C)	21.7	14.9	21	13.2	21.8	10.7	17.2	17.9	18.2	11.3	16.9	18.3

Table 2. Influence of a nontreated control (NTC), one application of tebuconazole (T (1x)), one application of tebuconazole + one application of soil surfactant (T (1x) + SS (1x)), one application of tebuconazole + 0.6 cm of post-application irrigation (T (1x) + PAI), one application of tebuconazole + one application of soil surfactant + 0.6 cm of post-application irrigation (T (1x) + SS (1x) + PAI), two applications of tebuconazole (T (2x)), two applications of tebuconazole + two applications of soil surfactant (T (2x) + SS (2x)), two applications of tebuconazole + 0.6 cm of post-application irrigation (T (2x) + PAI), and two applications of tebuconazole + two applications of soil surfactant + 0.6 cm of post-application irrigation (T (2x) + SS (2x) + PAI) on spring dead spot patch number and percent spring dead spot at Independence Golf Club, Midlothian, VA.

Treatment[1]	28 Apr 2020		21 May 2020		10 Jun 2020		27 Apr 2021		16 May 2021		07 Jun 2021	
	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS
NTC	1.75[4]	1.50	1.50	1.00	1.25	0.53	7.25	8.33	7.00	6.10	1.50	2.15
T[2] (1x)	1.00	0.25	0.25	0.03	0.00	0.00	2.00	2.28	1.25	1.10	0.50	0.75
T (1x) + SS[3] (1x)	0.25	0.03	0.75	0.58	1.00	0.28	3.25	4.13	2.25	2.35	1.25	1.33
T (1x) + PAI	1.00	1.25	0.75	0.40	0.75	0.50	1.50	1.83	0.75	0.74	1.00	1.18
T (1x) + SS (1x) + PAI	0.75	0.53	0.50	0.50	1.00	0.49	4.50	5.10	2.75	1.85	0.00	0.00
T (2x)	0.50	0.33	0.25	0.13	0.25	0.09	2.50	2.05	1.00	0.63	0.00	0.00
T (2x) + SS (2x)	0.50	0.13	0.25	0.13	0.50	0.18	4.75	5.30	2.00	1.95	0.00	0.00
T (2x) + PAI	0.50	0.50	0.50	0.23	0.25	0.15	1.75	2.40	2.25	1.39	0.25	0.05
T (2x) + SS (2x) + PAI	0.50	0.50	0.25	0.08	0.00	0.00	2.25	2.75	1.00	0.83	0.75	0.70
P-Value	0.6201	0.2569	0.2012	0.2200	0.1522	0.2085	0.2666	0.3238	0.0974	0.0822	0.2328	0.3075

[1]Tebuconazole rate = 1.5 kg ai ha^{-1}; single soil surfactant application rate = 50.9 L ha^{-1}; split soil surfactant application rate = 25.5 L ha^{-1}; irrigation amount = 0.6 cm

[2]Tebuconazole 3.6 F [Quali-Pro, Houston, TX]

[3]Hydra-Last [Landscape Supply Inc., Roanoke, VA]

[4]Data were subjected to ANOVA and means were compared when appropriate within assessment date using Tukey's Honest Significant Difference test (P = 0.05). There were no significant differences at this location, so no connecting letters report is provided.

Table 3. Influence of a nontreated control (NTC), one application of tebuconazole (T (1x)), one application of tebuconazole + one application of soil surfactant (T (1x) + SS (1x)), one application of tebuconazole + 0.6 cm of post-application irrigation (T (1x) + PAI), one application of tebuconazole + one application of soil surfactant + 0.6 cm of post-application irrigation (T (1x) + SS (1x) + PAI), two applications of tebuconazole (T (2x)), two applications of tebuconazole + two applications of soil surfactant (T (2x) + SS (2x)), two applications of tebuconazole + 0.6 cm of post-application irrigation (T (2x) + PAI), and two applications of tebuconazole + two applications of soil surfactant + 0.6 cm of post-application irrigation (T (2x) + SS (2x) + PAI) on spring dead spot patch number and percent spring dead spot at Nutters Crossing Golf Club, Salisbury, MD.

Treatment[1]	6 May 2020		28 May 2020		16 Jun 2020		5 May 2021		26 May 2021	
	Patch #	% SDS	Patch #	% SDS	Patch #	% SDS	Patch #	% SDS	Patch #	% SDS
NTC	4.25[4]	2.75	-	7.50	1.00	0.69	-	23.75	-	6.00
T[2] (1x)	2.25	1.63	-	4.50	1.00	0.68	-	33.75	-	14.25
T (1x) + SS[3] (1x)	2.00	2.13	-	5.00	0.75	0.75	-	34.50	-	13.25
T (1x) + PAI	2.25	1.19	-	1.25	0.50	0.20	-	27.00	-	8.75
T (1x) + SS (1x) + PAI	2.00	1.80	-	1.75	0.00	0.00	-	43.75	-	19.25
T (2x)	0.75	0.55	-	0.50	0.00	0.00	-	32.00	-	15.75
T (2x) + SS (2x)	1.75	0.68	-	1.25	0.00	0.00	-	46.25	-	26.50
T (2x) + PAI	0.75	0.55	-	1.50	0.00	0.00	-	38.75	-	16.25
T (2x) + SS (2x) + PAI	1.75	1.88	-	2.25	0.00	0.00	-	30.00	-	8.25
P-Value	0.3897	0.4514	-	0.0613	0.1065	0.2128	-	0.4941	-	0.3474

[1]Tebuconazole rate = 1.5 kg ai ha^{-1}; single soil surfactant application rate = 50.9 L ha^{-1}; split soil surfactant application rate = 25.5 L ha^{-1}; irrigation amount = 0.6 cm

[2]Tebuconazole 3.6 F [Quali-Pro, Houston, TX]

[3]Hydra-Last [Landscape Supply Inc., Roanoke, VA]

[4]Data were subjected to ANOVA and means were compared when appropriate within assessment date using Tukey's Honest Significant Difference test (P = 0.05). There were no significant differences at this location, so no connecting letters report is provided.

Table 4. Influence of a nontreated control (NTC), one application of tebuconazole (T (1x)), one application of tebuconazole + one application of soil surfactant (T (1x) + SS (1x)), one application of tebuconazole + 0.6 cm of post-application irrigation (T (1x) + PAI), one application of tebuconazole + one application of soil surfactant + 0.6 cm of post-application irrigation (T (1x) + SS (1x) + PAI), two applications of tebuconazole (T (2x)), two applications of tebuconazole + two applications of soil surfactant (T (2x) + SS (2x)), two applications of tebuconazole + 0.6 cm of post-application irrigation (T (2x) + PAI), and two applications of tebuconazole + two applications of soil surfactant + 0.6 cm of post-application irrigation (T (2x) + SS (2x) + PAI) on spring dead spot patch number and percent spring dead spot at Virginia Tech Turfgrass Research Center, Blacksburg, VA.

Treatment[1]	22 May 2020		05 Jun 2020		22 Jun 2020		15 May 2021		03 Jun 2021		22 Jun 2021	
	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS	Patch #	%SDS
NTC	6.75a[4]	4.88a	5.25a	2.85	2.50	1.70	15.75a	12.83a	15.25a	10.63a	17.00a	10.83a
T[2] (1x)	1.75b	1.00b	1.00ab	0.45	0.75	0.35	9.00ab	6.75ab	7.25ab	5.55ab	6.50b	4.58ab
T (1x) + SS[3] (1x)	1.50b	1.08b	1.00ab	0.50	0.75	0.58	6.00bc	3.25b	5.50b	3.88ab	4.50b	2.13b
T (1x) + PAI	3.00b	1.45b	1.50ab	0.55	1.00	0.29	8.50bc	5.80ab	6.50ab	4.35ab	6.00b	3.80ab
T (1x) + SS (1x) + PAI	2.25b	1.50b	1.50ab	1.73	1.25	0.98	4.25bc	2.63b	2.50b	2.06b	7.00b	2.89ab
T (2x)	2.00b	1.70b	1.25ab	0.58	0.00	0.00	3.00bc	1.80b	1.75b	0.83b	3.00b	1.55b
T (2x) + SS (2x)	0.50b	0.10b	0.00b	0.00	0.00	0.00	4.00bc	3.88ab	2.75b	3.08ab	3.25b	2.81ab
T (2x) + PAI	2.00b	0.88b	3.50ab	2.75	1.50	0.88	1.75c	1.10b	1.25b	0.70b	3.25b	0.70b
T (2x) + SS (2x) + PAI	1.25b	1.00b	0.25b	0.10	0.00	0.00	2.50bc	2.58b	1.75b	1.08b	1.25b	0.70b
P-Value	<0.0001	0.0016	0.0268	0.1053	0.1116	0.0739	<0.0001	0.0062	0.0004	0.0056	0.0003	0.0131

[1]Tebuconazole rate = 1.5 kg ai ha^{-1}; single soil surfactant application rate = 50.9 L ha^{-1}; split soil surfactant application rate = 25.5 L ha^{-1}; irrigation amount = 0.6 cm

[2]Tebuconazole 3.6 F [Quali-Pro, Houston, TX]

[3]Hydra-Last [Landscape Supply Inc., Roanoke, VA]

[4]Data were subjected to ANOVA and means were compared when appropriate within assessment date using Tukey's Honest Significant Difference test (P = 0.05). Columns within the same assessment date with similar letters are not significantly different.

Chapter 5: Fungicide Application Timing Model for Spring Dead Spot based on Soil Temperature and Season

Abstract

Spring dead spot (SDS) (*Ophiosphaerella* spp.) is the most detrimental disease to warm-season turfgrasses in the transition zone of the United States. Fungicides are often applied in the fall to prevent symptoms the following spring. However, fungicide applications do not provide consistent SDS suppression. One potential reason for this inconsistency is the use of calendar-based fungicide applications instead of a more targeted approach of using both time of year and soil temperature to optimize fungicide application timing. A field study was conducted at three separate hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) locations in Virginia to determine the optimal soil temperature and timing for SDS suppression with tebuconazole and isofetamid. One location was naturally infested with *O. korrae*, another was naturally infested with *O. herpotricha*, and a third location was infested with a mixture of both species due to natural populations and artificial inoculation. Tebuconazole (1.5 kg a.i. ha^{-1}) and isofetamid (4.1 kg a.i. ha^{-1}) were applied at 11 different timings throughout the year based on soil temperatures at a 0 to 10 cm depth. Plots were assessed for SDS severity three times in the spring and early summer of 2021. Two *in vitro* studies were also conducted with *O. herpotricha* and *O. korrae* isolates to determine 1) the optimal temperature for growth on potato dextrose agar (PDA) placed on a thermogradient table (13 to 33°C) and 2) the rate of growth of *O. herpotricha* and *O. korrae* isolates at 11, 19, and 27.5°C on PDA. In the field study, isofetamid suppressed SDS more than tebuconazole at all locations. Only the fall applications of isofetamid between 12.8 and 21.1°C soil temperatures suppressed SDS compared to the nontreated control at the *O. korrae* location. Moreover, fall treatments of isofetamid at soil temperatures between 4.4 and 21.1°C generally

provided the greatest SDS suppression at the *O. herpotricha* location. Fungicide treatments did not differ from the nontreated control at the mixed *Ophiosphaerella* species inoculation site. For the *in vitro* studies, both species grew optimally between 24 and 25°C, *O. korrae* displayed a greater growth rate at 11°C, and *O. herpotricha* displayed a greater growth rate at 27.5°C.

Introduction

Spring dead spot (SDS) (*Ophiosphaerella* spp.) is a challenging turfgrass disease to manage. It occurs on warm-season turfgrasses, particularly bermudagrass (*Cynodon* spp.), in regions where the turf is exposed to freezing temperatures during winter dormancy (Tredway et al., 2009). Symptoms appear as isolated patches that are sunken, circular, straw-colored, and necrotic ranging from 0.2 to 1 m in diameter at spring greenup (Wadsworth and Young, 1960; Lucas, 1980). The sunken nature of the patches reduces playability and safety on athletic fields, golf courses, and home lawns. Moreover, the patches are aesthetically displeasing (Beck et al., 2012, p. 7; Miller et al., 2017). Effective management of SDS is inconsistent and challenging (Tredway et al., 2009).

Cultural management practices targeting SDS have been widely studied, yet the research has yielded mixed results. Fall potassium applications reduced SDS in some studies and increased the disease in other studies (Cottrill et al., 2016; Dernoeden et al., 1991; McCarty et al., 1992). The effects of nitrogen source have been variable on SDS with ammonium sulfate or calcium nitrate reducing SDS in some studies while having no effect in others (Cottrill et al., 2016; Dernoeden et al., 1991; Tredway et al., 2020). Aggressive cultivation practices such as vertical mowing + core aeration or fraze mowing are effective at removing thatch and preventing SDS, but aggressive cultivation during the spring and early summer does not increase bermudagrass recovery from SDS damage (Hutchens et al., 2022b; Miller et al., 2017; Tisserat and Fry, 1997). Similar to cultural management practices, research efforts focused on chemical suppression of SDS have yielded erratic results. The effectiveness of fungicide applications for SDS are based primarily on the efficacy of the fungicide and the general timing of the fungicide application.

Numerous fungicides across a variety of fungicide groups have been tested *in vitro* and *in situ* against *Ophiosphaerella* spp. The demethylation inhibitors (DMIs), particularly tebuconazole, have been used for SDS management (Butler and Tredway, 2006; Tredway et al., 2009; Walker, 2006; Walker, 2009). Tebuconazole is efficacious *in vitro* against *Ophiosphaerella* spp. with ≤ 0.36 mg kg^{-1} suppressing mycelial growth by half across eight different *O. herpotricha* and *O. korrae* isolates (Hutchens et al., 2019b). However, in field studies tebuconazole provided inconsistent suppression of SDS (Earlywine and Miller, 2012; Hutchens et al., 2020; Tredway et al., 2007; Tredway et al., 2020). Tredway et al. (2007) found excellent suppression of SDS with tebuconazole while Booth et al. (2021) and Earlywine and Miller (2012) found no reduction in SDS severity with tebuconazole. The *Ophiosphaerella* species present is likely a major contributor to differential efficacy of tebuconazole against SDS with *O. herpotricha* being more sensitive to the fungicide than *O. korrae* (Tredway et al., 2020).

The recently developed fungicide, isofetamid, has consistently provided excellent suppression of SDS. Isofetamid applied twice in the fall at a rate of 2.04 kg ai ha^{-1} reduced SDS area under the disease progress curve (AUDPC) > 99% (Stephens et al., 2020). A Galle et al. (2019) study determined isofetamid rates as low as 0.64 kg ai ha^{-1} applied twice in the late summer/fall reduced SDS patch area > 84% compared to a nontreated control. Isofetamid also suppressed SDS to an acceptable level at both *O. korrae* and mixed *O. herpotricha* and *O. korrae* populations suggesting that the fungicide is effective, regardless of *Ophiosphaerella* species (Hutchens et al., 2020). The EC_{50} values for isofetamid on *O. herpotricha* and *O. korrae* isolates were ≤ 0.21 mg L^{-1} providing further support for the expansive efficacy of the fungicide (Hutchens et al., 2019b).

Accurate fungicide application timing is crucial for SDS management (Butler and Tredway, 2006; Lucas, 1980; Walker, 2006; Walker, 2009). Fungicides are most effective when the pathogen is active because mycelia are more likely to absorb the fungicide through the hyphal tips (Latin, 2021, p. 41). The optimal *in vitro* growth on potato dextrose agar (PDA) of *O. herpotricha* and *O. korrae* is reported to be 20 to 25°C, yet the optimal temperature for colonization and infection to cause the most severe symptoms is 15 to 17°C (Caasi et al., 2010; Crahay et al., 1988; Flores et al., 2015; Perry et al., 2010; Tisserat et al., 1989; Walker et al., 2006). No studies have been conducted showing the growth rate of *O. herpotricha* and *O. korrae* at a range of temperatures, which could influence how the pathogens infect the plant and how fungicides are absorbed by the pathogens (Latin, 2021, p. 41; Magarey et al., 2005).

Whether SDS should be treated with fungicide during optimal temperature for *Ophiosphaerella* spp. growth or during optimal temperature for hybrid bermudagrass infection and damage is not clear. Studies have shown that fall fungicide applications are generally the most efficacious against SDS, yet spring applications, alone, are not effective (Butler and Tredway, 2006; Lucas, 1980; Walker, 2006; Walker, 2009). Lucas (1980) showed that benomyl applications in October, November, and December provided complete suppression of SDS while applications during the spring, summer, and winter were ineffective at suppressing SDS. Walker (2006, 2009) showed that fall fungicide applications, as well as spring + fall applications, were most efficacious against SDS. Butler and Tredway (2006) determined that fall fenarimol applications were effective against SDS. The aforementioned studies focused on calendar-based fungicide applications with only the Walker (2006) study reporting soil temperatures at application. Our goals were to determine both optimal time of year and soil temperature for SDS fungicide applications, further

elucidate optimal temperature for *O. herpotricha* and *O. korrae in vitro* growth, and determine the rate of growth of *O. herpotricha* and *O. korrae* at a range of temperatures *in vitro*.

Materials and Methods

In Vitro Optimal Temperature for Growth Study

A study was conducted in February of 2019 and repeated in March of 2019 to determine the optimal temperature for growth *in vitro* of four *O. herpotricha* isolates and four *O. korrae* isolates. Plugs (6-mm diameter) from each isolate were placed onto potato dextrose agar (PDA) [Difco Laboratories Inc., Franklin Lakes, NJ, USA] at a concentration of 39 g^{-L} in Petri dishes. Plates were sealed with parafilm, inverted, and placed onto a thermogradient table in the dark with surface temperatures ranging from 13 to 33°C (Welbaum et al., 2016). There were 15 columns across the thermogradient table, and each isolate was placed randomly within each column to capture a range of temperatures across the thermogradient table. Within-column temperature variability was minimal (±1°C). Surface temperatures were measured directly under each plate with a Craftsman 50455 near-infrared thermometer [Stanley Black & Decker, New Britain, CT, USA]. The isolates were allowed to grow for 14 days then mycelial diameters were measured (mm) in two directions and averaged. Mycelial diameters of the four isolates of each species were pooled, plotted, and a Gaussian peak model (Peak Value x Exp [- (0.5 x ((Temperature (°C) – Critical Point) / Growth Rate)^2)]) was fit using JMP Pro 15 [Statistical Analysis Software, Cary, NC, USA] to determine the optimal temperature for growth of *O. herpotricha* and *O. korrae*.

In Vitro Rate of Growth Study

An additional *in vitro* study was conducted with a single *O. herpotricha* and *O. korrae* isolate to determine the rate of growth of the two species at three different temperatures (11, 19, and 27.5°C). Plugs (7-mm diameter) of each isolate were plated onto PDA contained in Petri

dishes. The plates were sealed with parafilm, inverted, and incubated in the dark at either 11, 19, or 27.5°C. Mycelial diameters were measured in two directions and averaged 0, 5, 10, 15, and 20 days after plating.

Data were sorted by isolate and temperature and plotted in JMP Pro 15 with day as the independent variable (*x*-axis) and mycelial growth as the dependent variable (*y*-axis). A line was then fit using linear regression for each replication and slope was determined for each replication to show the rate of growth in mm^{-day}. There were four replications, and the study was repeated. The slopes from the linear regression were subjected to ANOVA, the two runs were pooled together ($P = 0.3259$) and means of the slopes for each isolate x temperature were compared using a Student's *t*-test ($P \leq 0.05$) in JMP Pro 15.

Field Fungicide Application Timing Study

A trial to determine the optimal application timing of tebuconazole (1.5 kg a.i. ha^{-1}) [Torque, Nufarm, Melbourne, Australia] and isofetamid (4.1 kg a.i. ha^{-1}) [Kabuto, PBI Gordon, Shawnee, KS, USA] was conducted from the spring of 2020 to the summer of 2021 at three hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) locations in Virginia. The trial was conducted on a 'Tifway 419' golf course fairway mowed at a height of 16 mm in Chesterfield, VA, a 'Northbridge' golf course fairway mowed at a height of 25 mm in Blacksburg, VA, and a 'Patriot' research area maintained like a golf course fairway or athletic field mowed at a height of 15 mm at the Virginia Tech Turfgrass Research Center, Blacksburg, VA. Only *O. korrae* was isolated from the Chesterfield location according to a Hutchens et al. (2021) study. Samples were not taken from the Blacksburg location, but data from the Hutchens et al. (2021) paper suggest that *O. herpotricha* is the likely causal agent at that location due to geographic proximity to nearby sampling sites. Lastly, at the Virginia Tech Turfgrass Research Center

location, 70% of the samples had *O. herpotricha* and 20% of the samples had *O. korrae* according to the Hutchens et al. (2021) study. Moreover, the Virginia Tech Turfgrass Research Center location was also inoculated with both *O. herpotricha* and *O. korrae* isolates, so the authors assume that this location had a mixed *Ophiosphaerella* population for the duration of the trial.

Tebuconazole and isofetamid were applied at various timings based on season and five-day average soil temperature at a 0 to 10-cm depth throughout the year (Table 1). Five-day average soil temperatures were monitored using the Syngenta GreenCast online application [https://www.greencastonline.com/tools/soil-temperature, Syngenta, Basel, Switzerland]. Certain timing treatments (e.g., summer 26.7°C treatment) were not applied at the Blacksburg and Virginia Tech Turfgrass Research Center locations because five-day average soil temperatures never reached the required temperatures for those treatments. Fungicide treatments were applied with a CO_2-pressurized sprayer at 276 kPa of pressure and a carrier volume of 814 L ha^{-1}. At Chesterfield and the Virginia Tech Turfgrass Research Center, all treatments were irrigated in post-application except for the two wintertime treatments because the irrigation systems were already winterized. The Blacksburg location was a non-irrigated site; however, some applications were made prior to rainfall.

The study was set up as a randomized complete block design with three replications at the Chesterfield location and four replications at the Blacksburg and Virginia Tech Turfgrass Research Center locations. Plots were assessed visually three times from initial greenup in the beginning of the spring of 2021 to the summer of 2021 for percent SDS. Space limitations required that the study be repeated over the following year on the same plots, so the spring treatment plots were treated for the second year during the assessment period for the first year which may have had a mild effect on the hybrid bermudagrass recovery from the disease. Only data from the first year of

the study are presented in this manuscript. Area under the disease progress curves (AUDPCs) were determined for percent SDS data, subjected to analysis of variance (ANOVA), and means were separated using a Student's *t*-test ($P \leq 0.05$) in JMP Pro 15.

Results

In Vitro Optimal Temperature for Growth Study

The optimal temperature for growth of the four *O. herpotricha* isolates fit into the same Gaussian peak model was 24.8°C ($r^2 = 0.93$) (Fig. 1). The optimal temperature for growth of the four *O. korrae* isolates fit into the same Gaussian peak model was 24.4°C ($r^2 = 0.89$). The model also determined peak mycelial growth of *O. herpotricha* and *O. korrae* to be 64.6 and 68.8 mm, respectively.

In Vitro Rate of Growth Study

Linear regression was effective at modeling isolate growth rate over time ($r^2 \geq 0.87$). The main effect of temperature was the most significant effect for the *in vitro* rate of growth study ($P < 0.0001$; $F = 222.5851$) (Table 2). The *O. herpotricha* and *O. korrae* isolates pooled together had a greater growth rate at 19°C than 11 or 27.5°C (data not presented). The main effect of species was also significant ($P = 0.0027$; $F = 10.3743$) with *O. korrae* having an 8% greater growth rate than *O. herpotricha* when all temperatures were pooled together within species and species were compared (data not shown). The interaction effect of species x temperature was also significant ($P < 0.0001$; $F = 21.0792$). The means comparisons between the species x temperature combinations are presented in Figure 2. Both the *O. herpotricha* and *O. korrae* isolates had the greatest growth rate (> 3.1 mm^{-day}) at 19°C (Fig. 2). Both species had a > 27% greater growth rate at 19°C than the two species at 11 or 27.5°C. The *O. korrae* isolate at 11°C grew with a 32% higher growth rate than the *O. herpotricha* isolate at 11°C; moreover, *O. herpotricha* grew with a 14% higher growth

rate at 27.5°C than at 11°C. *Ophiosphaerella korrae* at 27.5°C had a similar growth rate to *O. herpotricha* at 11°C.

Field Fungicide Application Timing Study

At the Chesterfield, VA location (*O. korrae*), only the fall (21.1°C), fall (18.3°C), and late fall (12.8°C) isofetamid treatments reduced percent SDS AUDPC (> 92%) compared to the nontreated control (Fig. 3). No tebuconazole treatments provided SDS suppression at the Chesterfield, VA location. However, the summer (26.7°C), early fall (23.9°C), fall (21.1°C), fall (18.3°C), late fall (12.8°C), and late winter (4.4°C) isofetamid treatments and the spring (18.3°C), fall (18.3°C), and late fall (12.8°C) tebuconazole treatments all provided similar percent SDS AUDPCs to the fall (21.1°C), fall (18.3°C), and late fall (12.8°C) isofetamid treatments. The fall (21.1°C) isofetamid treatment provided greater SDS suppression than any of the spring isofetamid treatments.

At the Blacksburg, VA location (*O. herpotricha*), the early fall (23.9°C), fall (21.1°C), fall (18.3°C), late fall (12.8°C), late fall (7.2°C), and early winter (4.4°C) isofetamid applications all suppressed SDS by > 55% compared to the nontreated control (Fig. 4). The early fall (23.9°C), fall (18.3°C), late fall (12.8°C), and late winter (4.4°C) tebuconazole treatments suppressed SDS. Similar to isofetamid, these four tebuconazole treatments also reduced percent SDS AUDPC by > 55% compared to the nontreated control. The fall (21.1°C), fall (18.3°C), late fall (12.8°C), late fall (7.2°C), and early winter (4.4°C) isofetamid treatments reduced percent SDS AUDPC by > 76% compared to the early spring (15.6°C) and late spring (21.1°C) isofetamid applications. Additionally, the early fall (23.9°C), fall (18.3°C), late fall (12.8°C), and late winter (4.4°C) tebuconazole treatments reduced percent SDS AUDPC by > 57% compared to the spring (18.3°C) tebuconazole application.

At the Virginia Tech Turfgrass Research Center location (mixed *Ophiosphaerella* species), no fungicide application timing treatment was different than the nontreated control (Fig. 5). However, the fall (21.1°C), fall (18.3°C), late fall (12.8°C), late fall (7.2°C), and early winter (4.4°C) isofetamid applications had > 84% lower percent SDS AUDPC compared to the early summer (23.9°C) isofetamid treatment. Additionally, the fall (21.1°C) tebuconazole treatment reduced percent SDS AUDPC by > 71% compared to the early summer (23.9°C) and late fall (7.2°C) tebuconazole treatments. The tebuconazole and isofetamid treatments resulted in similar percent SDS AUDPCs when compared to each other at each application timing with the exception of the late fall (7.2°C) application timing in which the isofetamid treatment reduced percent SDS AUDPC by 86% compared to the tebuconazole treatment at that application timing.

Discussion

Our data showed that *O. herpotricha* and *O. korrae* grew optimally at 24.9°C and 24.6°C, respectively, *in vitro*, which is similar to previous reports (Crahay et al, 1988; Perry et al., 2010; Tisserat et al., 1989). Our study was different from previous studies in that each plate had a unique temperature producing continuous data that allowed for a finer resolution determination of optimal temperature for growth of *O. herpotricha* and *O. korrae*. Moreover, we found that the growth rates for *O. herpotricha* and *O. korrae* were similar at 19°C and 27.5°C, yet *O. korrae* had a greater growth rate at 11°C than *O. herpotricha*—this has not been reported. This result is not entirely surprising as the cause of necrotic ring spot on Kentucky bluegrass (*Poa pratensis* L.) is *O. korrae*, and necrotic ring spot has been reported as far north as British Columbia, Canada showing that the adaptive range of *O. korrae* expands well into cold, northern climates while *O. herpotricha* has not been reported as far north as Canada (MacDonald, 1990; MacDonald et al., 1991; Raffle and Hsiang, 1998). The ability of *O. korrae* to grow at a higher rate than *O. herpotricha* at cooler

temperatures *in vitro* also offers a potential reason for why *O. korrae* is generally less affected by fungicides than *O. herpotricha* in the field. *Ophiosphaerella korrae* may be able to recover from fall fungicide suppression and colonize and infect the turfgrass more rapidly than *O. herpotricha* during the cool late fall and winter months. However, isolation of both *O. herpotricha* and *O. korrae* from bermudagrass roots has been reported to be prevalent during late fall for both species suggesting that colonization of bermudagrass by both species during cool fall and winter months is likely, but no study has been conducted directly comparing bermudagrass colonization by the two species during various times of year and soil temperatures (Perry et al., 2010; Walker et al., 2006).

Our field fungicide application timing study showed that isofetamid suppressed SDS more than tebuconazole at each location when all application timing treatments were pooled by fungicide and compared (data not shown). These results are similar to previous results when tebuconazole and isofetamid efficacy against SDS were compared in the same field study (Hutchens et al., 2020). Tebuconazole is not consistently efficacious against SDS, regardless of application timing, while isofetamid has been proven highly efficacious in multiple studies (Earlywine and Miller, 2012; Galle et al., 2019; Hutchens et al., 2020; Stephens et al., 2020; Tredway et al., 2007; Tredway et al., 2020). One reason for the inconsistent efficacy of tebuconazole could be due to its high affinity to bind to organic carbon (K_{oc}) making the fungicide less likely to reach the pathogen in the underground portions of the plant (Hutchens et al., 2019a; Hutchens et al., 2022a).

The application timing window of isofetamid for suppression of SDS at the *O. korrae* location (Chesterfield, VA) was smaller than for the *O. herpotricha* location (Blacksburg, VA), but fall applications, particularly of isofetamid, were generally more effective at suppressing SDS

than applications made at any other time of year. The efficacy of fall timing is consistent with previous literature. In previous studies, fall applications of benomyl and propiconazole were effective at suppressing SDS, yet spring applications, alone, did not suppress SDS compared to a nontreated control (Lucas, 1980; Walker, 2006; Walker, 2009). At the *O. korrae* location (Chesterfield, VA), only the fall (21.1°C), fall (18.3°C), and late fall (12.8°C) treatments of isofetamid suppressed SDS compared to the nontreated control. Moreover, the only tebuconazole treatments to suppress SDS at any location were the fall (18.3°C), late fall (12.8°C), and the late winter (4.4°C) application timings at the *O. herpotricha* (Blacksburg, VA) location—the late winter (4.4°C) application was the only tebuconazole application timing not in the fall that was effective against SDS.

Lucas (1980) and Walker (2009) showed that spring fungicide applications, alone, did not suppress SDS. Our study also showed that spring applications of isofetamid or tebuconazole were not effective suggesting that soil temperature, alone, is not sufficient for optimizing efficacy of fungicide applications for SDS—applying at the proper soil temperature and time of year are both necessary. For the more efficacious fungicide, isofetamid, time of year (fall) was more important than applying at a specific soil temperature during the fall as all fall isofetamid applications resulted in similar percent SDS, regardless of location. However, with tebuconazole, the late fall (7.2°C) application at the Virginia Tech Turfgrass Research Center resulted in greater percent SDS than the other fall applications showing that tebuconazole applications at different soil temperatures within the fall season differentially affected efficacy against SDS. Previous research has shown how fungicide application based on calendar date affects fungicide efficacy against SDS, but our study was the first to show how both season and soil temperature during application influence fungicide efficacy against SDS (Lucas, 1980; Walker, 2006; Walker, 2009). Our

recommendation for turfgrass professionals managing SDS is to apply a highly efficacious fungicide such as isofetamid one to two times during the fall months when 10-cm depth soil temperatures are at five-day averages of 13-21°C or when five-day average soil temperatures are from 13-18°C for less efficacious products like tebuconazole.

References

Beck, L.L. 2012. Evaluation of chemical and cultural methods for the management of spring dead spot in bermudagrass turf. PhD diss. Texas Tech University, Lubbock, TX. p. 7.

Booth, J.C., Sullivan, D., Askew, S.A., Kochersberger, K., and McCall, D.S. 2021. Investigating targeted spring dead spot management via aerial mapping and precision-guided fungicide applications. Crop Sci. 61:3134-3144. https://doi.org/10.1002/csc2.20623

Butler, E.L. and Tredway, L.P. 2006. Method and Timing of Fungicide Applications for Control of Spring Dead Spot in Hybrid Bermudagrass. Online. Plant Health Prog. doi:10.1094/PHP-2006-0901-01-RS.

Caasi, O.C., Walker, N.R., Marek, S.M., Enis, J.N., and Mitchell, T.K. 2010. Infection and colonization of turf-type bermudagrass by *Ophiosphaerella herpotricha* expressing green or red fluorescent proteins. Phytopathology 100:415-423. doi:10.1094/PHYTO-100-5-0415.

Crahay, J.N., Dernoeden, P.H., and O'Neill, N.R. 1988. Growth and pathogenicity of *Leptosphaeria korrae* in bermudagrass. Plant Dis. 72:945-949.

Cottrill, D.J., Earlywine, D.T., and Miller, G.L. 2016. Assessment of nitrogen source, sulfur, and fall fungicide applications on the management of spring dead spot of bermudagrass. Plant Dis. 100:473-482. doi:http://dx.doi.org/10.1094/PDIS-05-15-0565-RE

Dernoeden, P.H., Crahay, J.N., and Davis, D.B. 1991. Spring Dead Spot and Bermudagrass Quality As Influenced by Nitrogen Source and Potassium. Crop Sci. 31:1674-1680.

Earlywine, D.T. and Miller, G.L. 2012. Evaluation of fungicides for spring dead spot control on bermudagrass, 2010-2011. Plant Dis. Manag. Rep. 6:T022.

Flores, F.J., Marek, S.M., Anderson, J.A., Mitchell, T.K., and Walker, N.R. 2015. Infection and colonization of several bermudagrasses by *Ophiosphaerella korrae*. Phytopathology 105:656-661. http://dx.doi.org/10.1094/PHYTO-07-14-0205-R

Galle, G.H., Kerns, J.P., Butler, E.L., and Ploetz, J.N. 2019. Evaluation of Kabuto and Tekken for the control of spring dead spot on ultradwarf bermudagrass putting greens, 2017-2018. Plant Dis. Manag. Rep. 13:T006.

Hutchens, W.J., Booth, J.C., Doherty, J.R., Roberts, J.A., and McCall, D.S. 2022a. Influence of post-application irrigation and soil surfactants on tebuconazole efficacy against spring dead spot. Crop Pro. 156. https://doi.org/10.1016/j.cropro.2022.105961

Hutchens, W.J., Booth, J.C., Goatley, J.M., and McCall, D.S. 2022b. Cultural and Fertility Practices Influence Hybrid Bermudagrass Recovery from Spring Dead Spot Damage. HortSci. 57:332-336. https://doi.org/10.21273/HORTSCI16235-21

Hutchens, W.J., Gannon, T.W., Shew, H.D., and Kerns, J.P. 2019a. Effect of post-application irrigation on fungicide movement and efficacy against *Magnaporthiopsis poae*. Crop Prot. 122:106-111. https://doi.org/10.1016/j.cropro.2019.04.027

Hutchens, W.J., Henderson, C.A., Bush, E.A., Kerns, J.P., and McCall, D.S. 2021. Geographic Distribution of *Ophiosphaerella* Species in the Mid-Atlantic United States. Plant Health Prog. https://doi.org/10.1094/PHP-04-21-0076-S

Hutchens, W.J., Nagaoka, Y., Kerns, J. P., Goatley, J. M., Nita, M., & McCall, D. S. 2019b. Variable sensitivity of *Ophiosphaerella* spp. causing spring dead spot to fungicides and temperature. Paper 120074. Paper presented at the annual meeting of ASA, CSSA, and SSSA, San Antonio, TX.

Hutchens, W.J., Nagaoka, Y., Kerns, J.P., Goatley, J.M., Nita, M., Booth, J.C., and McCall, D.S. 2020. Differential Response of *Ophiosphaerella* Species *In Situ* to Various Fungicides. Crop Science Society of America Annual Meeting (Virtual).

Latin, R. 2021. A Practical Guide to Turfgrass Fungicides, 2nd ed. APS Press, St. Paul, MN. p. 41.

Lucas, L.T. 1980. Control of spring dead spot of bermudagrass with fungicides in North Carolina. Plant Dis. 64:868-870.

MacDonald, L.S. 1990. 1989 vegetable, turf and ornamental diseases in British Columbia. Can. Plant Dis. Surv. 70:34-35.

MacDonald, L.S., Deyoung, R., and Omrod, D.J. 1991. Turfgrass diseases diagnosed at the B.C. Ministry of Agriculture and Fisheries Plant Diagnostic Laboratory in 1990. Can. Plant Dis. Surv. 71:128.

Magarey, R.D., Sutton, T.B., and Thayer, C.L. 2005. A simple generic infection model for foliar fungal plant pathogens. Phytopathology 95:92-100. doi:10.1094/PHYTO-95-0092.

McCarty, L.B., Lucas, L.T., and DiPaola, J.M. 1992. Spring Dead Spot Occurrence in Bermudagrass following Fungicide and Nutrient Applications. HortSci. 27.10:1092-1093.

Miller, G.L., Earlywine, D.T., and Fresenburg, B.S. 2017. Effect of Fraze Mowing on Spring Dead Spot Caused by *Ophiosphaerella herpotricha* of Bermudagrass. Int. Turfgrass Soc. Res. J. 13:225-228. doi:10.2134/itsrj2016.10.0839

Perry, D.H., Tomaso-Peterson, M., and Baird, R. 2010. Seasonal variation in frequency of isolation of *Ophiosphaerella korrae* from bermudagrass roots in Mississippi and pathogenicity and optimal growth of selected isolates. Mycopathologia 169:395-402. doi:10.1007/s11046-010-9273-x

Raffle, V.R., and Hsiang, T. 1998. Low level of DNA polymorphisms in isolates of *Leptosphaeria korrae* pathogenic on *Poa pratensis*. Can. J. Plant Pathol. 20:48-54.

Stephens, C.M., Ploetz, J.N., Butler, E.L., and Kerns, J.P. 2020. Evaluation of fungicides for the control of spring dead spot on ultradwarf bermudagrass putting greens, 2018-2019. Plant Dis. Manag. Rep. 14:T011.

Tisserat, N.A., Pair, J.C., and Nus, A. 1989. *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermudagrass in Kansas. Plant Dis. 73:933-937.

Tisserat, N.A. and Fry, J.D. 1997. Cultural practices to reduce spring dead spot (*Ophiosphaerella herpotricha*) severity in *Cynodon dactylon*. J. Intl. Turf. Res. 8:931-936.

Tredway, L.P., Butler, E.L., and Soika, M.D. 2007. Evaluation of fungicides for preventative control of spring dead spot in bermudagrass, 2005-2006. Plant Dis. Manag. Rep. 1:T030.

Tredway, L.P., Soika, M.D., Butler, E.L., and Kerns, J.P. 2020. Impact of nitrogen source, fall fertilizers, and preventative fungicides on spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha*. Crop Sci. Special Issue: International Turfgrass Research Conference: 1-10. doi:10.1002/csc2.20306

Tredway, L. P., Tomaso-Peterson, M., Perry, H., and Walker, N. R. 2009. Spring dead spot of bermudagrass: A challenge for researchers and turfgrass managers. Online. Plant Health Prog. doi:10.1094/PHP-2009-0710-01-RV.

Wadsworth, D. F. and Young, H. C. 1960. Spring dead spot of bermudagrass. Plant Dis. 44:516-518.

Walker, N.R. 2006. Evaluation of timing and application frequency of tebuconazole for the management of spring dead spot of bermudagrass, 2004-2005. Plant Dis. Manag. Rep. 1:T051.

Walker, N.R. 2009. Influence of fungicide application timings on the management of bermudagrass spring dead spot caused by *Ophiosphaerella herpotricha*. Plant Dis. 93:1341-1345.

Walker, N.R., Mitchell, T.K., Morton, A.N., and Marek, S.M. 2006. Influence of temperature and time of year on colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. Plant Dis. 90:1326-1330. https://doi.org/10.1094/PD-90-1326

Welbaum, G.E., Khan, O.S., and Samarah, N.H. 2016. A Gusseted Thermogradient Table to Control Soil Temperatures for Evaluating Plant Growth and Monitoring Soil Processes. J. Vis. Exp. 116:e54647. doi:10:3791/54647

Table 1. Five-day average soil temperatures on day of application and date of application for fungicide efficacy trials at Chesterfield, VA (infested with *Ophiosphaerella korrae*), Blacksburg, VA (infested with *O. herpotricha*), and the Virginia Tech Turfgrass Research Center (VTTRC) (mixed *Ophiosphaerella* infestations) are demonstrated.

Treatment[1]	**Chesterfield, VA (*O. korrae*)**		**Blacksburg, VA (*O. herpotricha*)**		**VTTRC (Mixed)**	
	Soil Temp. (°C)	*Application Date*	*Soil Temp. (°C)*	*Application Date*	*Soil Temp. (°C)*	*Application Date*
Early spring (15.6°C)	14.9[2]	8 Apr 2020	15.7	19 May 2020	15.7	19 May 2020
Spring (18.3°C)	13.1	14 May 2020	18.2	4 Jun 2020	18.2	2 Jun 2020
Late spring (21.1°C)	21.3	3 Jun 2020	20.9	11 Jun 2020	20.9	11 Jun 2020
Early summer (23.9°C)	24.7	11 Jun 2020	N/A	N/A	24.2	20 Jul 2020
Summer (26.7°C)	26.3	7 Jul 2020	N/A	N/A	N/A	N/A
Early fall (23.9°C)	26.4	28 Aug 2020	24.2	20 Jul 2020	N/A	N/A
Fall (21.1°C)	19.1	21 Sep 2020	21.3	14 Sep 2020	21.3	14 Sep 2020
Fall (18.3°C)	18.3	28 Sep 2020	16.7	21 Sep 2020	16.7	21 Sep 2020
Late fall (12.8°C)	14.8	17 Nov 2020	13.2	8 Oct 2020	13.2	8 Oct 2020
Late fall (7.2°C)	N/A[3]	N/A	6.7	23 Nov 2020	6.7	23 Nov 2020
Early winter (4.4°C)	5.2	8 Jan 2021	5.6	14 Dec 2020	5.6	14 Dec 2020
Late winter (4.4°C)	12.3	16 Mar 2021	5.9	1 Mar 2021	5.9	1 Mar 2021

[1]Application timing treatments for isofetamid applied at 4.1 kg a.i. ha^{-1} and tebuconazole applied at 1.5 kg a.i. ha^{-1}

[2]All actual five-day average soil temperatures were within ± 2.5°C of the soil temperatures designated in the treatment list except for the early spring (15.6°C) and late winter (4.4°C) applications at the Chesterfield, VA location.

[3]Certain summer and early fall treatments were missed at the Blacksburg, VA and Virginia Tech Turfgrass Research Center locations and are designated with N/A.

Table 2. Analysis of variance for the main effects and interactions for the influence of temperature on growth rate of a *Ophiosphaerella herpotricha* and *O. korrae* isolate *in vitro*.

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
Run	1	0.000088	0.000088	0.0019	0.9651
Temperature	2	20.127954	10.063977	222.5851	<0.0001
Species	1	0.469063	0.469063	10.3743	0.0027
Run*Temperature	2	0.038404	0.019202	0.4247	0.6572
Run*Species	1	0.021042	0.021042	0.4654	0.4995
Temperature*Species	2	1.906154	0.953077	21.0792	<0.0001
Temperature*Species*Run	2	0.104600	0.0523	1.1567	0.3259
Error	36	1.627706	0.04521		
Total	47	24.295012			

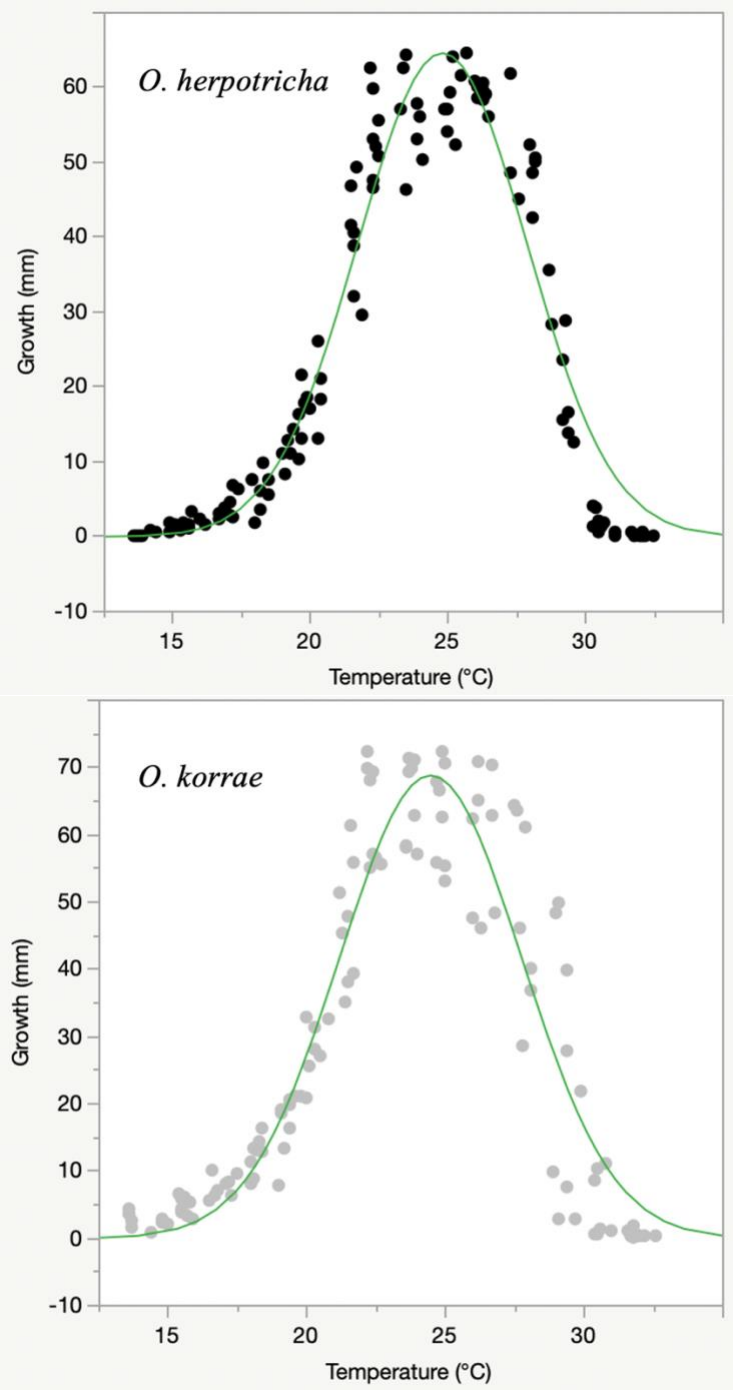

Figure 1. Optimal temperature for growth of four *Ophiosphaerella herpotricha* isolates (top) (24.9°C; $r^2 = 0.93$) and four *O. korrae* isolates (bottom) (24.6°C; $r^2 = 0.89$). A Gaussian peak model was fit to the data and optimal temperatures for growth were determined based on the critical point (peak) of the model.

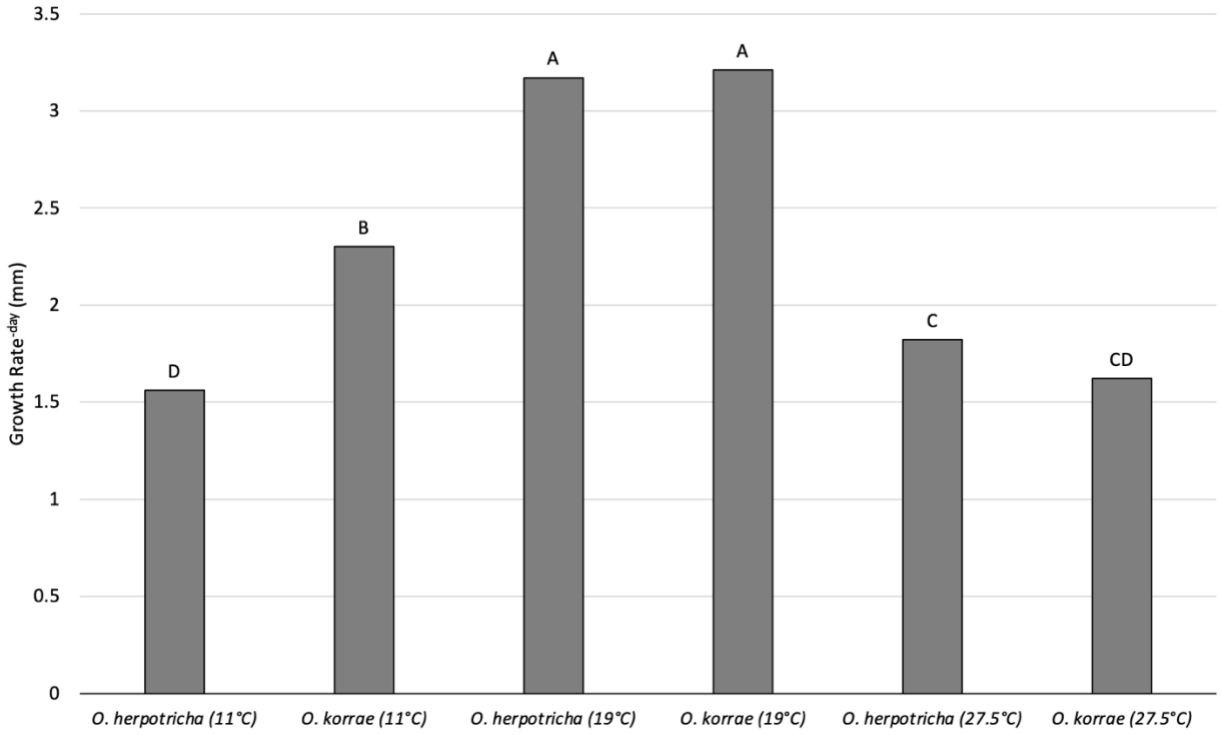

Figure 2. The influence of temperature on growth rate (mm^{-day}) of an *Ophiosphaerella herpotricha* and *O. korrae* isolate. Data were subjected to analysis of variance and means were separated with a Student's *t*-test ($P \leq 0.05$). Columns with similar letters are not significantly different.

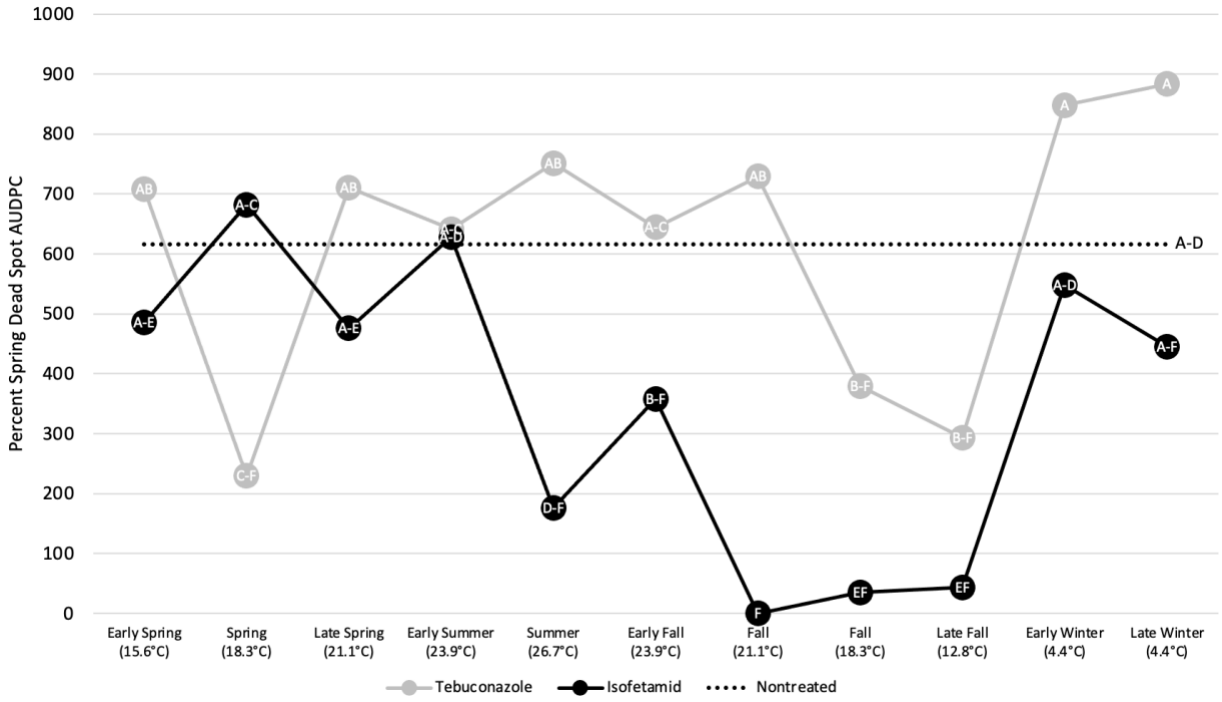

Figure 3. The influence of application timing of isofetamid (4.1 kg a.i. ha^{-1}) and tebuconazole (1.5 kg a.i. ha^{-1}) on percent spring dead spot area under the disease progress curve (AUDPC) at the Chesterfield, VA (*O. korrae*) location. Treatments with similar letters are not significantly different according to a Student's *t*-test (P = 0.05).

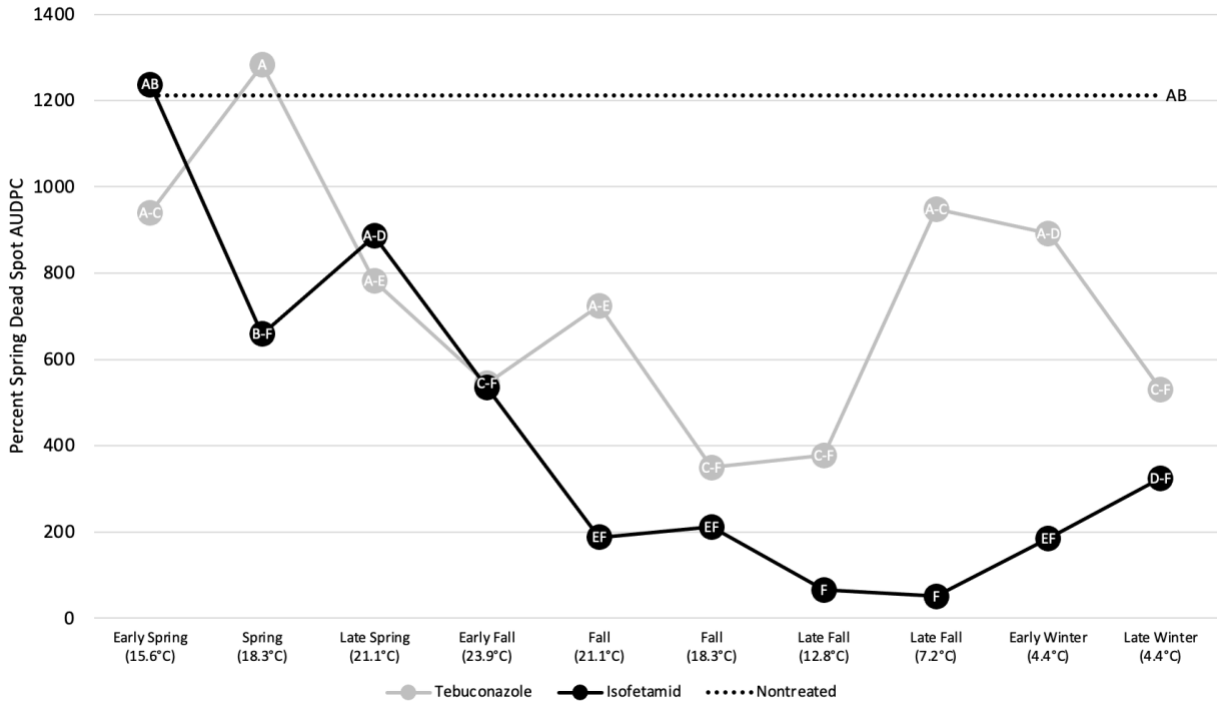

Figure 4. The influence of application timing of isofetamid (4.1 kg a.i. ha^{-1}) and tebuconazole (1.5 kg a.i. ha^{-1}) on percent spring dead spot area under the disease progress curve (AUDPC) at the Blacksburg, VA (*O. herpotricha*) location. Treatments with similar letters are not significantly different according to a Student's *t*-test (P = 0.05).

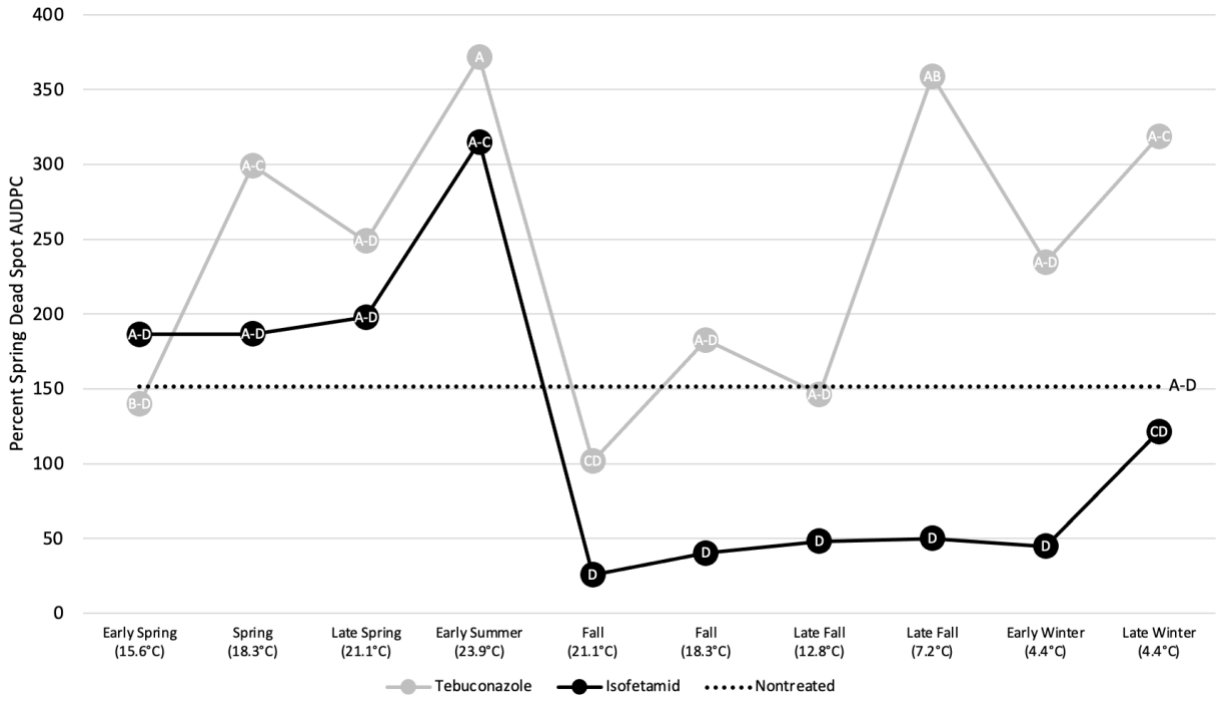

Figure 5. The influence of application timing of isofetamid (4.1 kg a.i. ha^{-1}) and tebuconazole (1.5 kg a.i. ha^{-1}) on percent spring dead spot area under the disease progress curve (AUDPC) at the Virginia Tech Turfgrass Research Center (mixture of *O. herpotricha* and *O. korrae*) location. Treatments with similar letters are not significantly different according to a Student's *t*-test (P = 0.05).

Chapter 6: Environmental and Edaphic Factors that Influence Spring Dead Spot Epidemics

Abstract

Spring dead spot (*Ophiosphaerella* spp.) is a soilborne disease of warm-season turfgrasses in areas where winter dormancy occurs. The environmental and edaphic factors that influence where spring dead spot epidemics occur are not well defined. A study was conducted in the spring of 2020 and repeated during the spring of 2021 on four 'TifSport' hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) golf course fairways expressing spring dead spot symptoms in Cape Charles, VA, USA. Spring dead spot within each fairway was mapped from aerial imagery collected in the spring of 2019 with a 20 MP CMOS 4k true color sensor mounted on a DJI Phantom 4 Pro drone. Three disease intensity zones were designated from the maps (low, moderate, high) using the Jenks Natural Breaks classification method based on the density of spring dead spot patches in an area. Disease incidence and severity, soil samples, compaction, thatch, and organic matter measurements were taken from ten plots within each disease intensity zone from each of the four fairways (n=120). Multivariate pairwise correlation analysis ($P < 0.1$) was conducted to determine which edaphic and environmental factors most influenced the spring dead spot epidemic. Moreover, stepwise regression was conducted to select variables for an optimum model for each hole by year. Edaphic factors that correlated with an increase in spring dead spot or were selected for the best fitting model varied across holes and years. However, increases in soil pH and thatch depth were two commonly selected predictors for an increase in spring dead spot. These results will allow turfgrass managers to effectively implement targeted chemical and cultural practices where spring dead spot pressure is most intense.

Introduction

Spring dead spot (SDS) (*Ophiosphaerella* spp.) epidemics occur frequently on hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) in the transition zone of the United States. Spring dead spot epidemics can reduce the quality of bermudagrass surfaces (Tredway et al., 2009). Spring dead spot appears as circular, sunken, and necrotic patches as bermudagrass breaks winter dormancy and begins to green up in the springtime (Wadsworth and Young, 1960). The disease is caused by three soilborne fungal species within the genus *Ophiosphaerella*: *O. herpotricha* (Fr:Fr) J. Walker, *O. korrae* (J. Walker & A.M. Smith) Shoemaker & C.E. Babcock (=*Leptosphaeria korrae* J. Walker & A.M. Smith), and *O. narmari* (J. Walker & A.M. Smith) Wetzel, Hulbert, & Tisserat (=*L. narmari* J. Walker & A.M. Smith) (Flores et al., 2017; Iriarte et al., 2004; Iriarte et al., 2005; Wetzel et al., 1999). In Virginia, SDS is caused predominantly by *O. herpotricha* and *O. korrae* (Hutchens et al., 2021).

On a microscale, the host-pathogen interaction between *Ophiosphaerella* spp. and hybrid bermudagrass is well understood, yet the spatial components of SDS epidemics on a broader scale are not documented (Caasi et al., 2010; Flores et al., 2015). Plant disease epidemics can have three distinct distribution patterns: regular, random, or aggregated/clumped/clustered (Campbell and Noe, 1985; Madden et al., 2007; Xu and Ridout, 1998). Typically, soilborne diseases have an aggregated spatial distribution, but this is not documented for SDS (Campbell and Benson, 1994; Campbell and Noe, 1985). The spatial distribution of SDS epidemics warrants further research.

Edaphic factors often influence the occurrence of soilborne disease symptoms in certain areas (Campbell and Benson, 1994; Campbell and Noe, 1985; Noe and Barker, 1985; Pair et al., 1986). Pair et al. (1986) showed that SDS patches are associated more with heavier soils than sandier soils suggesting that soil type or soil bulk density/compaction could be a driving factor in

SDS epidemics. Lucas (1980) also suggested that soil compaction was associated with SDS development, but Martin et al. (2001) reported that little research has been done on the influence of soil compaction on SDS. Other researchers have studied the effects of nutrients and pH on SDS symptoms in small plots and *Ophiosphaerella* spp. growth *in vitro* (Cottrill et al., 2016; Tredway et al., 2020). However, a broad-scale study has not been conducted to determine the influence of various edaphic factors on the spatial distribution of SDS epidemics.

The influence of nutrients on SDS have been widely studied with mixed results. McCarty et al. (1992) and Perry et al. (2010) showed an increase in SDS with sulfur-coated urea and sulfur applications, respectively. However, Cottrill et al. (2016) found that SDS severity was reduced with sulfur applications, yet Tredway et al. (2020) found no effect of sulfur on SDS severity. These results suggest that sulfur may have an influence on SDS, but it is not clear how much of an effect or if the influence is positive or negative toward SDS. Tredway et al. (2020) showed that *O. herpotricha* and *O. korrae* respond differently to certain nutrient applications. The authors determined that ammonium sulfate and calcium nitrate differentially suppressed *O. herpotricha* and *O. korrae* with ammonium sulfate suppressing *O. herpotricha* and calcium nitrate suppressing *O. korrae* (Tredway et al., 2020). However, Cottrill et al. (2016) determined that both *O. herpotricha* and *O. korrae* grew more on calcium nitrate-amended media than ammonium sulfate-amended media in an *in vitro* study. Potassium chloride applications were beneficial for SDS reduction in a study by Dernoeden et al. (1991), yet potassium sulfate applications increased SDS in a McCarty et al. (1992) study. Manganese was associated with higher SDS severity in a Perry et al. (2010) study in which SDS was caused by *O. korrae*. Similarly, Tredway et al. (2020) found a positive correlation with foliar manganese content and SDS severity when the disease was caused

by *O. korrae*. These data suggest that manganese may be an influential nutrient on SDS epidemics (Perry et al., 2010; Tredway et al., 2020).

The influence of pH on SDS has produced mixed results as well. Vincelli et al. (2021) suggested that maintaining a more acidic soil pH of 5.0 to 5.3 would reduce SDS pressure, yet Tredway et al. (2020) determined that there was a significant positive correlation with soil pH and SDS symptoms caused by *O. herpotricha* and a significant negative correlation with soil pH and SDS symptoms caused by *O. korrae*. Cottrill et al. (2016) reported that *O. herpotricha* grew optimally *in vitro* at a pH range of 5 to 6 while *O. korrae* grew optimally at a pH of 6, and both species grew less at a pH range of 7 to 9. Though the effect of soil pH on SDS is not fully understood, evidence suggests that it is an influential factor in the development and severity of the disease.

High thatch levels have been reported to increase pathogen inoculum densities in turfgrass systems (Allan-Perkins et al., 2018). McAfee (1979) reported that thatch contributes to SDS problems. Other authors suggested thatch accumulation is generally associated with SDS development (Lucas, 1980; Martin et al., 2001). Therefore, thatch is likely an influential factor in SDS epidemics.

Spring dead spot damage is caused from both the infection of *Ophiosphaerella* spp. and low temperatures during the winter (Booth et al., 2021; Tredway et al., 2009; Walker et al., 2006). Therefore, management practices that insulate the plant during cold temperatures could reduce damage caused by *Ophiosphaerella* spp., and irrigation prior to extreme cold temperatures could be a method of insulating the plant and reducing SDS damage. Irrigation has been shown to insulate fruit crops during cold temperatures (Tsipourdis et al., 2006; Wilcox and Davies, 1981). Moisture from irrigation events prior to frost increased citrus canopy temperatures and reduced

frost damage to the foliage (Wilcox and Davies, 1981). Irrigation can also increase canopy temperatures in other fruit crops such as cherries and peaches (Tsipourdis et al., 2006). So, areas with low soil moisture may predispose bermudagrass to greater SDS damage during cold temperatures, which has been reported anecdotally, yet areas with adequate soil moisture may reduce SDS damage. In contrast, greater soil moisture has been documented to favor soilborne diseases (Campbell and Benson, 1994).

Though many researchers have highlighted individual edaphic factors that affect SDS occurrence and severity, no field-scale multivariate study highlighting the influence of numerous edaphic factors on SDS has been conducted. The goal of our study was to determine the environmental and edaphic factors that influence SDS epidemics.

Materials and Methods

Aerial Image Collection, Processing, and Disease Map Construction

A study was conducted from 2019 to 2021 on four 'TifSport' hybrid bermudagrass golf course fairways (Holes 2, 3, 5, and 7) with a history of SDS epidemics in Cape Charles, VA, USA. Initial true color aerial images were collected from each fairway at an altitude of 45 m with a 20 MP CMOS 4k sensor mounted on a Phantom 4 Pro drone [DJI, Nanshan, Shenzhen, China] on 28 May 2019. Flight missions were created using the waypoint navigation software DroneDeploy [DroneDeploy, v 4.40.0, San Francisco, CA, USA] allowing for both autonomous flight and image collection. Images were taken with a 75% front overlap and a 70% overlap between images following the methods of Henderson (2021). Eight to ten ground control points were placed on permanent fixtures (irrigation heads, drainage basins, etc.) on each fairway and geotagged with a Phoenix 300 differential GPS receiver [Raven Industries, Sioux Falls, SD, USA] prior to image collection for georeferencing of images. Aerial images were then mosaicked together (full

keypoints image scale; Point cloud generation: Optimal settings with image scale ½) in Pix4D [Prilly, Switzerland] to create maps of each individual fairway (Henderson, 2021).

Maps created from 2019 images were used as baseline maps to determine sampling locations in the spring of 2020 and 2021. Spring dead spot patches from the 2019 maps were marked manually by a trained turfgrass pathologist using the "marker" tool in ArcGIS [West Redlands, CA, USA]. Three disease intensity zones (low, moderate, high) based on disease incidence were determined using the Jenks Natural Breaks classification method to classify the different disease intensity zones (Chen et al., 2013; Straw et al., 2019). Ten sampling locations within each disease intensity zone for each fairway were then marked in ArcGIS. Coordinates generated from these sampling locations were then input into a Garmin Oregon 750t GPS tracker [Garmin Ltd., Olathe, KS, USA].

Plot Assessment and Sampling

PVC plots (2 m x 2 m) were placed on the sampling location coordinates (± 5 m) from the GPS tracker on the fairways in the spring of 2020 and 2021. Ground control points were placed in the same locations as 2019 prior to aerial image collection in 2020 and 2021. Flights for 2020 were conducted from 25 May to 29 May at 34 m in altitude and flights for 2021 were conducted from 17 May to 18 May at 34 m in altitude. Aerial images for 2020 and 2021 flights were collected using similar methods as 2019 except the flights were conducted at 34 m and there was 80% front overlap and 80% side overlap between images.

Immediately after aerial images were collected in 2020 and 2021, plots were visually assessed for patch number (disease incidence) and percent SDS. A single plug (3 x 10 x 18 cm) was then pulled with a soil profiler from an area of healthy, green turf from each plot. Thatch and organic layer were visually determined from the plug, and thatch and organic layer depth were

measured (mm) with a ruler (Fig. 1). Five soil compaction measurements were taken with both a FieldScout TruFirm Turf Firmness Meter (penetrometer) [Spectrum Technologies, Inc., Aurora, IL, USA] and a Clegg soil impact tester (Clegg) [SD Instrumentation Ltd., Tellisford, United Kingdom] within each plot and averaged in 2021—compaction measurements were not taken in 2020. Moreover, five points within each plot were measured for soil moisture (4-cm depth) and averaged in 2021 using a TDR 350 [Spectrum Technologies, Inc., Aurora, IL, USA]. The holes were not irrigated for at least 24 hours prior to sampling in 2021 to optimize relative soil moisture differences. Additionally, soil samples were taken within each plot by placing a soil probe (2-cm diameter) [JMC Soil Samplers, Newton, IA, USA] 10 cm into the ground. The top ~2.5 to 5 cm of organic material/thatch was then removed from the intact soil cores that were retrieved. The remaining material was then placed into sampling boxes. Ten soil subsamples were taken within each plot and homogenized to create one representative soil sample per plot.

Soil Sample Processing

The soil samples were air dried for > 48 h, sieved (2-mm mesh), and 10-g subsamples from each homogenized sample were processed using a Mehlich 1 extraction procedure. A total of 20 mL of double acid extracting solution (0.5 M HCl + 0.025 M H_2SO_4) was added to 5 g of each homogenized soil sample. Samples were then shaken (180 $cycles^{-min}$) for five minutes. After the samples were shaken, the solution was poured through filter paper [Whatman No. 4, Cytiva, Marlborough, MA, USA] into 50 mL centrifuge tubes. The centrifuge tubes containing the extract were then placed into cold storage (4°C) until further processing.

The following nutrients were extracted from the soil samples from the Mehlich 1 soil test: phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), boron (B), and aluminum (Al). Nutrient concentrations (mg L^{-1}) of the

extracts were determined using inductively coupled plasma atomic emission spectrometry [ICP-AES ARCOS, Spectro Analytical Instruments, Kleve, Germany]. Appropriate duplicates and blank samples (negative controls) were also processed to validate the accuracy of HPLC outputs. The soil pH was determined by placing a pH meter probe into a 1:1 ratio of soil and deionized water solution. Lastly, soil samples were oven dried for > 2 h and soil organic carbon weight (mg) was then determined via combustion with a high temperature oven set to 360°C for 2 h (Maguire and Heckendorn, 2019). Soil organic carbon percentage (% OM) was determined by subtracting the combusted sample weight from the oven-dried sample weight prior to combustion and dividing by the oven-dried sample weight prior to combustion.

Statistical Analysis

Data were compared using a multivariate pairwise correlation analysis in JMP Pro 15 [Statistical Analysis Software, Cary, NC, USA]. Data were sorted by fairway and year, and significant correlations ($P < 0.1$) were determined between each variable. Pearson's correlation coefficients (PCC) and significance levels between the SDS measurements (patch number and percent SDS) and the edaphic factors measured (soil compaction, soil moisture, thatch layer, organic layer, soil nutrient content, soil pH, and soil organic carbon) are presented. The data were then subjected to the best subset stepwise regression method to determine which variables resulted in the best or optimal model.

Results

Using pairwise correlation analysis, thatch was positively correlated with patch number two of eight site-years ($PCC \geq 0.3674$) and was negatively correlated with patch number once ($PCC = -0.3490$) (Figs. 2,3). Thatch was also a positive predictor variable for patch number using best subset stepwise regression analysis on Hole 5 in 2021 (Table 2). Moreover, there were

significant positive correlations between thatch and percent SDS for two of eight site-years (PCC ≥ 0.3398) (Figs. 4,5). Thatch was also a positive predictor variable for percent SDS for three of eight site-years (Tables 3,4).

Soil pH was consistently positively correlated with both patch number and percent SDS. There were positive correlations (PCC ≥ 0.4190) between soil pH and patch number for two of eight site-years and a positive correlation (PCC = 0.4767) between soil pH and percent SDS on Hole 3 in 2021 (Figs. 2,3,5). Moreover, soil pH was a positive predictor for both patch number and percent SDS two of eight site-years (Tables 1,3,4).

Potassium was positively correlated twice with patch number (PCC ≥ 3497) and once among the eight site-years with percent SDS (PCC = 0.5240) (Figs. 2-4). Potassium was also a positive predictor variable once for patch number and twice for percent SDS using the best subset stepwise regression model selection (Tables 2-4).

Phosphorus was generally positively correlated with SDS, but there were mixed results, particularly with patch number. Phosphorus was positively correlated with patch number two of eight site-years (PCC ≥ 3934) and also negatively correlated with patch number (PCC ≤ -0.3768) two of eight site-years (Figs. 2,3). For percent SDS, phosphorus was positively correlated three of eight site-years (PCC ≥ 0.3199) (Figs. 4,5). Moreover, phosphorus was a positive predictor for patch number twice and positive predictor for percent SDS once using the best subset stepwise regression analysis (Tables 1,2,4).

Both calcium and magnesium were generally negatively associated with SDS. Calcium (PCC = -0.3746) and magnesium (PCC = -0.3070) were negatively correlated with patch number on Hole 3 in 2021 (Fig. 3). Calcium was also negatively correlated (PCC = -0.3131) with percent SDS on Hole 3 in 2021 and magnesium was negatively correlated (PCC = -0.3313) with percent

SDS on Hole 5 in 2021 (Fig. 5). Magnesium was a negative predictor variable for both patch number and percent SDS a total of five times, however, in contrast, calcium was a positive predictor variable for patch number once (Tables 1-4).

Zinc, manganese, and copper were generally negatively associated with SDS. Zinc was negatively correlated once with patch number (PCC = -0.3148) and once with percent SDS (PCC = -0.3221) (Figs. 3,4). Moreover, manganese was negatively correlated with patch number (PCC = -0.3884) once and was a negative predictor variable for both patch number and percent SDS once (Fig. 3; Tables 2,4). Copper was positively correlated with percent SDS (PCC = 0.3413) on Hole 3 in 2021 (Fig. 5). In contrast, copper was a negative predictor variable for either patch number or percent SDS three times (Tables 2,4). Moreover, aluminum, boron, and iron had variable effects on SDS with boron being the most robust edaphic factor in its influence on SDS. Boron was generally negatively associated with SDS while aluminum and iron did not have a consistent positive or negative association with SDS.

Both percent soil organic matter and organic layer depth provided mixed results in their effect on SDS with both positive and negative correlations (Figs. 2-5). Moreover, organic layer was a negative predictor variable for patch number twice, yet percent soil organic matter was either a positive or negative predictor variable for SDS depending on the site-year (Tables 1-4). Additionally, surface hardness measurements with both the penetrometer and Clegg soil impact tester (Clegg) did not significantly correlate with patch number or percent SDS in the pairwise correlation analysis. However, for the stepwise regression best fitting model analysis, the penetrometer measurements were a positive predictor variable for patch number on Hole 7 in 2021 and for percent SDS on Hole 5 in 2021. Moreover, Clegg measurements were a positive predictor variable for percent SDS on Hole 3 in 2021. Lastly, percent soil moisture was negatively correlated

with percent SDS on Hole 5 (PCC = -0.3191) in 2021 (Fig. 5). In contrast, percent soil moisture was a positive predictor variable once for both patch number and percent SDS in 2021 (Tables 2,4).

Discussion

Our study produced mixed results regarding the effect of edaphic factors on SDS depending on the year and hole that was sampled. However, certain edaphic factors were consistently associated with SDS in both the pairwise correlation analyses and the stepwise regression best fitting model analyses. At the location where we conducted this research only *O. korrae* had been isolated, so more research needs to be conducted on the influence of edaphic factors on SDS at locations where *O. herpotricha* is the predominant species causing the disease.

Soil pH was generally positively associated with SDS in our study, which is different from what Tredway et al. (2020) observed with SDS caused by *O. korrae*. Tredway et al. (2020) reported a negative relationship between soil pH and SDS; however, soil pH ranged only from 4.65 to 5.84 in our study while it ranged from ~3.5 to ~7.0 in the Tredway et al. (2020) study, which may account for the different findings. Cottrill et al. (2016) determined that *O. korrae* grew increasingly more *in vitro* as pH increased from 4 to 6, which aligns with our findings that SDS increased with increasing soil pH with a range of 4.65 to 5.84.

Although soil pH was generally positively associated with SDS in our study, contrarily, magnesium and calcium concentrations in the soil were generally negatively associated with SDS. Tredway et al. (2020) showed a negative correlation between SDS caused by *O. korrae* and foliar calcium content, which aligns with our findings of a negative relationship between soil calcium content and SDS. Calcium may reduce pathogen infection thereby leading to less SDS (Kunoh, 1990; Tredway et al., 2020). Magnesium has also been reported to reduce certain plant diseases

(Huber and Jones, 2013). There was a positive relationship between soil pH and SDS and a negative relationship between soil magnesium and soil calcium content and SDS in our study. This suggests that the reduction of SDS from greater soil calcium and magnesium content are likely not related to soil pH. Instead, calcium and magnesium may have a direct inhibitory effect on *O. korrae* or increase the ability of the bermudagrass plant to survive from *O. korrae* infection.

We also observed a positive relationship between potassium and SDS in our study similar to what McCarty et al. (1992) reported. However, Dernoeden et al. (1991) found that potassium chloride reduced SDS while Tredway et al. (2020) found no association between potassium content and SDS. Potassium has been reported to be associated with predisposing peanuts to greater damage by *Rhizoctonia* and *Pythium* by reducing the calcium content of peanut pods (Huber and Jones, 2013). This suggests that increased potassium could lead to greater disease in certain scenarios. Additionally, phosphorus content in the soil had varying influences on SDS in our study, generally having a positive relationship with SDS. Tredway et al. (2020) found no significant correlation between SDS caused by *O. korrae* and foliar phosphorus content; however, they did observe a positive correlation between foliar phosphorus content and SDS caused by *O. herpotricha*.

Lower zinc and manganese content in the soil were generally associated with greater SDS in our study. Both zinc and manganese have been reported to reduce plant diseases such as coffee rust (*Hemileia vastatrix*) and tan spot in wheat (*Dreschlera tritici-repentis*), which aligns with our results (Simoglou and Dordas, 2006; Perez et al., 2020). Manganese and zinc can also have direct inhibitory effects on certain pathogens such as *Phytophthora nicotianae* (Luo et al., 2020). In contrast to our results, Tredway et al. (2020) found that foliar manganese content was positively correlated with SDS caused by *O. korrae*, and Perry et al. (2010) reported increased SDS severity

with manganese applications. No research on the influence of zinc on SDS has been reported until now. The other metals and metalloids (aluminum, boron, copper, and iron) had varying influences on SDS depending on the year and hole sampled. No prior research has reported a significant association of these elements in the soil with SDS. Future research should be conducted to elucidate the effects that each of the metals/metalloids have on SDS.

Surface firmness measured with both a FieldScout TruFirm Turf Firmness Meter (penetrometer) and a Clegg soil impact tester (Clegg) had marginal influence on SDS in our study. As surface firmness increases, Clegg readings increase and penetrometer readings decrease; in our study, the results were contradictory on Hole 3 in 2021 with both penetrometer and Clegg readings having a positive association with SDS. Our data suggest that the influence of surface firmness on SDS epidemics is inconclusive, which differs from the suggestion by Lucas (1980) that soil compaction could lead to greater SDS.

We observed a negative correlation between percent soil moisture and percent SDS on Hole 5 in 2021, which could potentially have been caused by reduced frost tolerance of the hybrid bermudagrass at lower moisture levels like what Wilcox and Davies (1981) observed on citrus. This negative correlation could also have been due to low soil moisture predisposing the plant to greater damage (Campbell and Benson, 1994). Additionally, water was withheld for two days longer on Hole 5 than the other holes sampled in 2021 due to an irrigation system issue, potentially exacerbating the relative soil moisture differences. In contrast to Hole 5 in 2021, there was a positive association in the model selection analysis between percent soil moisture and SDS patch numbers on Hole 7 in 2021 and percent SDS on Hole 3 in 2021. High soil moisture has been reported to be favorable for certain soilborne pathogens (Campbell and Benson, 1994).

The influence of percent organic matter in the soil and the organic layer depth produced mixed results in their associations with SDS in our study. However, generally, greater thatch depths were associated with greater SDS in our study. This supports what previous authors have suggested (Lucas, 1980; Martin et al., 2001; McAfee, 1979). Therefore, thatch management via practices such as topdressing, aerifying, vertical mowing, and/or fraze mowing could potentially suppress SDS epidemics over time.

Our data suggest that there are many factors that contribute to SDS development making it challenging to distinguish a single principal cause under real-world conditions across geospatial surfaces. Controlled environment studies are useful for direct comparison of specific variables of interest, but they do not address the challenges associated with confounding influences. More research needs to be conducted on how each factor we measured individually influences SDS in a controlled environment, particularly with the highly associated edaphic factors such as soil pH, potassium content, and thatch depth. Moreover, other factors that we did not measure such as topography and soil type should be tested for their influence on SDS epidemics as anecdotal evidence suggests they may affect SDS incidence and severity.

References

Allan-Perkins, E., Manter, D., and Jung, G. 2018. Abundance of Bacteria, Fungi, and *Sclerotinia homoeocarpa* in the Thatch and Soil of Golf Courses. Phytobiomes 2:71-81. https://doi.org/10.1094/PBIOMES-09-17-0036-R

Booth, J.C., Sullivan, D., Askew, S.A., Kochersberger, K., and McCall, D.S. 2021. Investigating targeted spring dead spot management via aerial mapping and precision-guided fungicide applications. Crop Sci. 61:3134-3144. https://doi.org/10.1002/csc2.20623

Caasi, O.C., Walker, N.R., Marek, S.M., Enis, J.N, and Mitchell, T.K. 2010. Infection and colonization of turf-type bermudagrass by *Ophiosphaerella herpotricha* expressing green or red fluorescent proteins. Phytopathology 100:415-423.

Campbell, C.L., and Benson, D.M. 1994. Epidemiology and Management of Root Diseases. Springer, Berlin, Heidelberg.

Campbell, C.L., and Noe, J.P. 1985. The Spatial Analysis of Soilborne Pathogens and Root Diseases. Ann. Rev. Phytopathol. 23:129-148.

Chen, J., Yang, S., Li, H., Zhang, B., and Lv, J. 2013. Research on geographical environment unit division based on the method of natural breaks (Jenks). ISPRS-International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences. Vol. XL-4/W3, p. 47.

Cottrill, D.J., Earlywine, D.T., and Miller, G.L. 2016. Assessment of nitrogen source, sulfur, and fall fungicide applications on the management of spring dead spot of bermudagrass. Plant Dis. 100:473-482. doi:http://dx.doi.org/10.1094/PDIS-05-15-0565-RE

Dernoeden, P.H., Crahay, J.N., and Davis, D.B. 1991. Spring dead spot and bermudagrass quality as influenced by nitrogen source and potassium. Crop Sci. 31:1674-1680.

Flores, F.J., Marek, S.M., Anderson, J.A., Mitchell, T.K., and Walker, N.R. 2015. Infection and colonization of several bermudagrasses by *Ophiosphaerella korrae*. Phytopathology 105:656-661.

Flores, F.J., Marek, S.M., Orquera, G., and Walker, N.R. 2017. Molecular Identification and Multilocus Phylogeny of *Ophiosphaerella* Species Associated with Spring Dead Spot of Bermudagrass. Crop Sci. 57:249-261. https://doi.org/10.2135/cropsci2016.05.0437

Henderson, C.A.T. 2021. Identification of Disease Stress in Turfgrass Canopies Using Thermal Imagery and Automated Aerial Image Analysis. Thesis. Virginia Tech University, Blacksburg, VA. Retrieved from https://vtechworks.lib.vt.edu/bitstream/handle/10919/103621/Henderson_CA_T_2021.pdf?sequence=1&isAllowed=y

Huber, D.M., and Jones, J.B. 2013. The role of magnesium in plant disease. Plant Soil 368:73-85. https://doi.org/10.1007/s11104-012-1476-0

Hutchens, W.J., Henderson, C.A., Bush, E.A., Kerns, J.P., and McCall, D.S. 2021. Geographic Distribution of *Ophiosphaerella* Species in the Mid-Atlantic United States. Plant Health Prog. https://doi.org/10.1094/PHP-04-21-0076-S

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., and Tisserat, N.A. 2004. Genetic diversity and aggressiveness of *Ophiosphaerella korrae*, a cause of spring dead spot of bermudagrass. Plant Dis. 88:1341-1346.

Iriarte, F.B., Wetzel, H.C., III, Fry, J.D., Martin, D.L., Vincelli, P., Dixon, E.W., and Tisserat, N.A. 2005. Aggressiveness of spring dead spot pathogens to bermudagrass. Int. Turf. Res. J. 10:258-264.

Kunoh, H. 1990. Ultrastructure and mobilization of ions near infection sites. Annu. Rev. Phytopathol. 28:93-111. https://doi.org/10.1146/annurev.py.28.090190.000521

Lucas, L.T. 1980. Spring dead spot of bermudagrass. p. 183-187. *In* P.O. Larsen and B.G. Joyner (ed.) Advances in turfgrass pathology. Harcourt Brace Jovanovich, Duluth, MN.

Luo, Y., Yao, A., Tan, M., Li, Z., Qing, L., and Yang, S. 2020. Effects of manganese and zinc on growth process of *Phytophthora nicotianae* and the possible inhibitory mechanisms. PeerJ. 8e8613. https://doi.org/10.7717/peerj.8613

Madden, L.V., Hughes, G., and van den Bosch, F. 2007. The Study of Plant Disease Epidemics. American Phytopathological Society, St. Paul, MN.

Martin, D.L., Bell, G.E., Baird, J.H., Taliaferro, C.M., Tisserat, N.A., Kuzmic, R.M., Dobson, D.D., and Anderson, J.A. 2001. Spring Dead Spot Resistance and Quality of Seeded Bermudagrasses under Different Mowing Heights. Crop Sci. 41:451-456.

McAfee, J. 1979. Proceedings of the Thirty-Fourth Annual Texas Turfgrass Conference p. 23-25.

McCarty, L.B., Lucas, L.T., and DiPaola, J.M. 1992. Spring dead spot occurrence in bermudagrass following fungicide and nutrient applications. HortSci. 27.10:1092-1093.

Noe, J.P., and Barker, K.R. 1985. Relation of within-field spatial variation of plant-parasitic nematode population densities and edaphic factors. Phytopathology 75:1247-1252.

Pair, J.C., Crowe, F.J., and Willis, W.G. 1986. Transmission of spring dead spot disease of bermudagrass by turf/soil cores. Plant Dis. 70:877-878.

Perry, D.H., Tomaso-Peterson, M., and Baird, R. 2010. Seasonal variation in frequency of isolation of *Ophiosphaerella korrae* from bermudagrass roots in Mississippi and pathogenicity and optimal growth of selected isolates. Mycologia 169:395-402. doi:10.1007/s11046-010-9273-x

Perez, C.D.P., Pozza, E.A., Pozza, A.A.A., Elmer, W.H., Pereira, A.B., Guimaraes, D.S.G., Monteiro, A.C.A., and Rezende, M.L.V. 2020. Boron, zinc and manganese suppress rust on coffee plants grown in a nutrient solution. Eur. J. Plant Pathol. 156:727-738. https://doi.org/10.1007/s10658-019-01922-9

Simoglou, K.B., and Dordas, C. 2006. Effect of foliar applied boron, manganese and zinc on tan spot in winter durum wheat. Crop Pro. 25:657-663. https://doi.org/10.1016/j.cropro.2005.09.007

Straw, C. M., Friell, J., & Horgan, B. 2019. Precision irrigation for golf courses using sensor and mapping technologies. Presented at the ASA, CSSA and SSSA International Annual Meetings, San Antonio, TX. November 2019.

Tredway, L.P., Soika, M.D., Butler, E.L., and Kerns, J.P. 2020. Impact of nitrogen source, fall fertilizers, and preventative fungicides on spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha*. Crop Sci. Special Issue: International Turfgrass Research Conference: 1-10. doi:10.1002/csc2.20306

Tredway, L. P., Tomaso-Peterson, M., Perry, H., and Walker, N. R. 2009. Spring dead spot of bermudagrass: A challenge for researchers and turfgrass managers. Online. Plant Health Progress doi:10.1094/PHP-2009-0710-01-RV.

Tsipourdis, C., Thomidis, T., and Xatzicharisis, I. 2006. Effect of sprinkler irrigation system on air temperatures and use of chemicals to protect cherry and peach trees from early spring frost. Aust. J. Exp. Agric. 46:697-700.

Vincelli, P. 2021. Managing Spring Dead Spot in Bermudagrass. PPFS-OR-T-13 University of Kentucky Cooperative Extension Service. http://plantpathology.ca.uky.edu/files/ppfs-or-t-13.pdf

Wadsworth, D. F. and Young, H. C. 1960. Spring dead spot of bermudagrass. Plant Dis. 44:516-518.

Walker, N.R., Mitchell, T.K., Morton, A.N., and Marek, S.M. 2006. Influence of temperature and time of year on colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. Plant Dis. 90:1326-1330.

Wetzel, H.C., III, Skinner, D.Z., and Tisserat, N.A. 1999. Geographic distribution and genetic diversity of three *Ophiosphaerella* species that cause spring dead spot of bermudagrass. Plant Dis. 83:1160-1166.

Wilcox, D., and Davies, F.S. 1981. Modification of Air Temperature and Citrus Leaf Temperature with High Volume Under-Tree Sprinklers. Proc. Fla. Hort. Soc. 94:59-63.

Xu, X.M., and Ridout, M.S. 1998. Effects of Initial Epidemic Conditions, Sporulation Rate, and Spore Dispersal Gradient on the Spatio-Temporal Dynamics of Plant Disease Epidemics. Phytopathology 88:1000-1012.

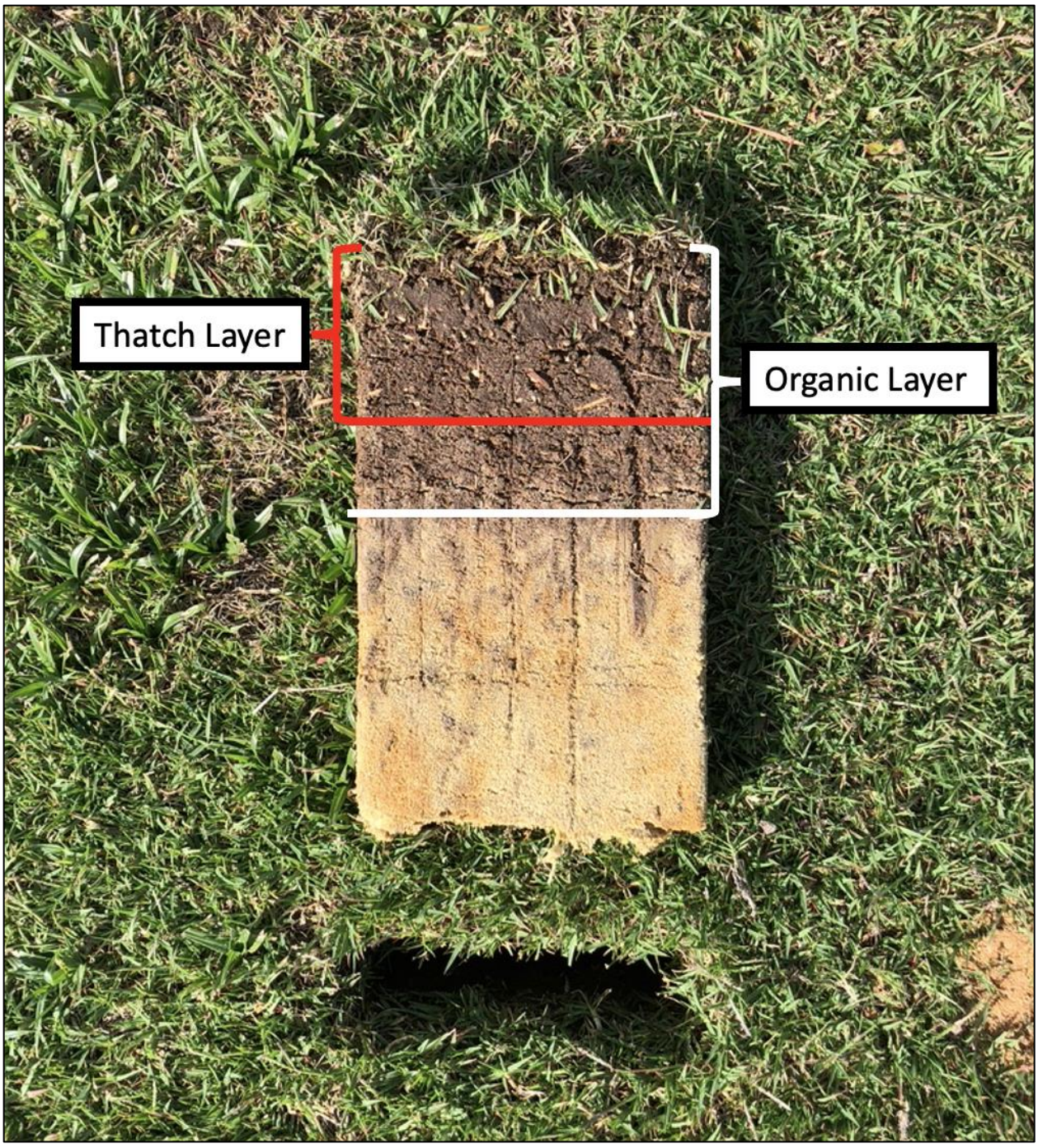

Figure 1. Thatch layer and organic layer were visually assessed. The bottom of the thatch layer was determined based on where the woody rhizomatous/stoloniferous material ended. The bottom of the organic layer was based on where the color changed from dark brown to light brown.

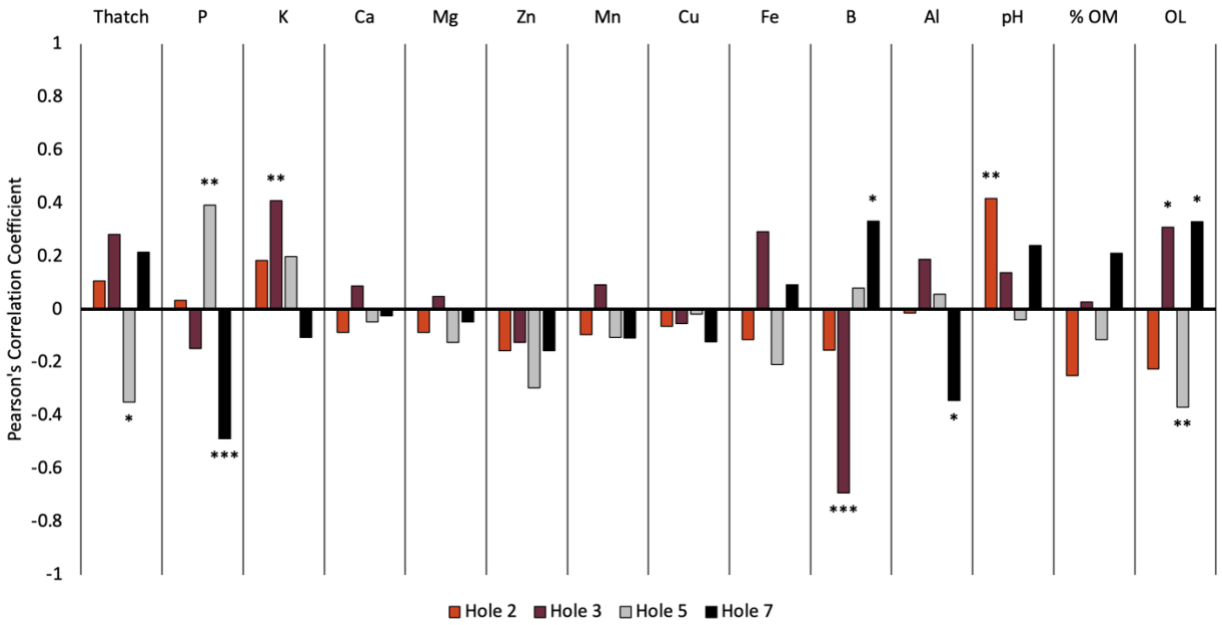

Figure 2. Pearson's correlation coefficients (PCC) and significance levels (* = P < 0.1; ** = P < 0.05; *** = P < 0.01) for spring dead spot patch number and various environmental and edaphic factors (% SM = percent soil moisture; Thatch = thatch depth; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Zn = zinc; Mn = manganese; Cu = copper; Fe = iron; B = boron; Al = aluminum; pH = soil pH; % OM = percent organic matter in soil; OL = organic layer depth for 2020.

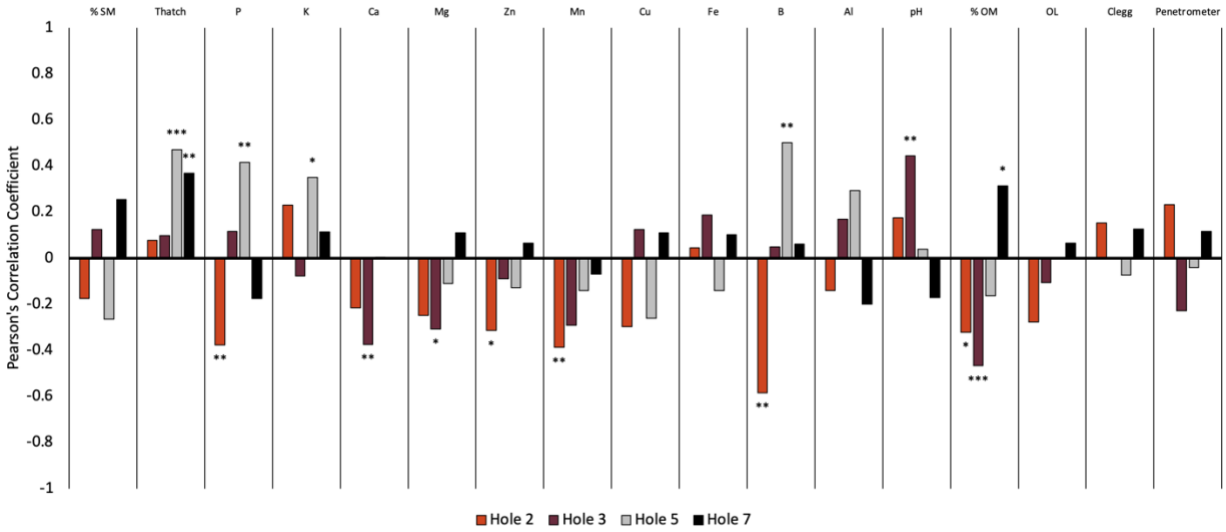

Figure 3. Pearson's correlation coefficients (PCC) and significance levels (* = P < 0.1; ** = P < 0.05; *** = P < 0.01) for spring dead spot patch number and various environmental and edaphic factors (% SM = percent soil moisture; Thatch = thatch depth; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Zn = zinc; Mn = manganese; Cu = copper; Fe = iron; B = boron; Al = aluminum; pH = soil pH; % OM = percent organic matter in soil; OL = organic layer depth; Clegg = surface hardness measured with a Clegg soil impact tester; Penetrometer = surface hardness measured with a turf firmness meter) for 2021.

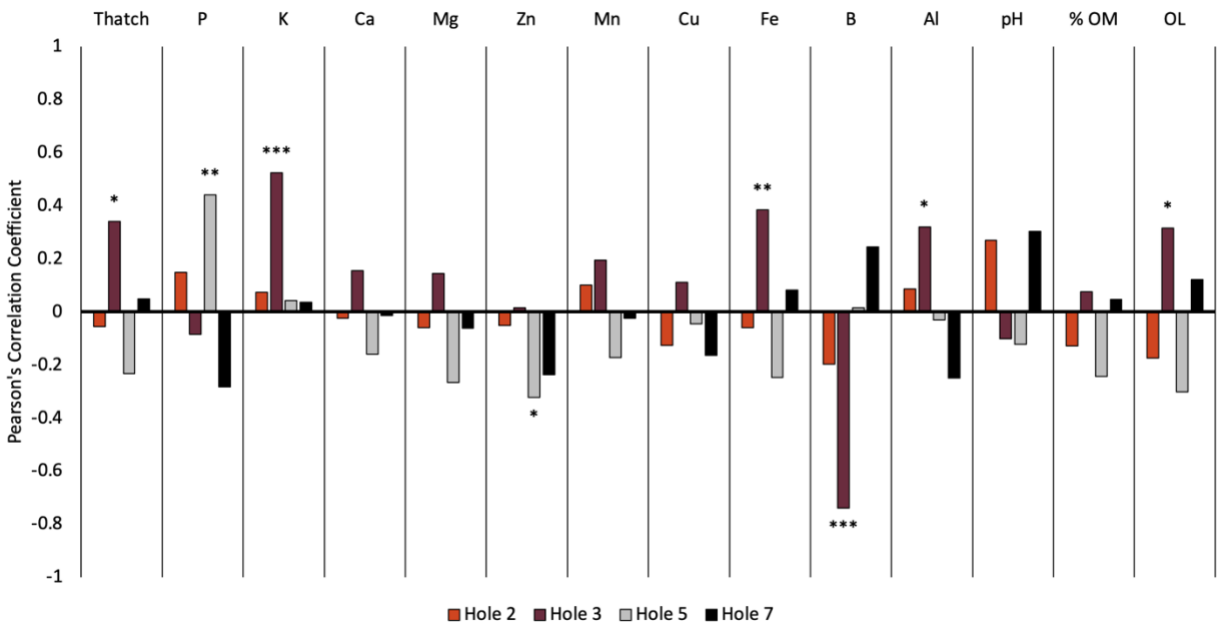

Figure 4. Pearson's correlation coefficients (PCC) and significance levels (* = P < 0.1; ** = P < 0.05; *** = P < 0.01) for percent spring dead spot and various environmental and edaphic factors (% SM = percent soil moisture; Thatch = thatch depth; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Zn = zinc; Mn = manganese; Cu = copper; Fe = iron; B = boron; Al = aluminum; pH = soil pH; % OM = percent organic matter in soil; OL = organic layer depth for 2020.

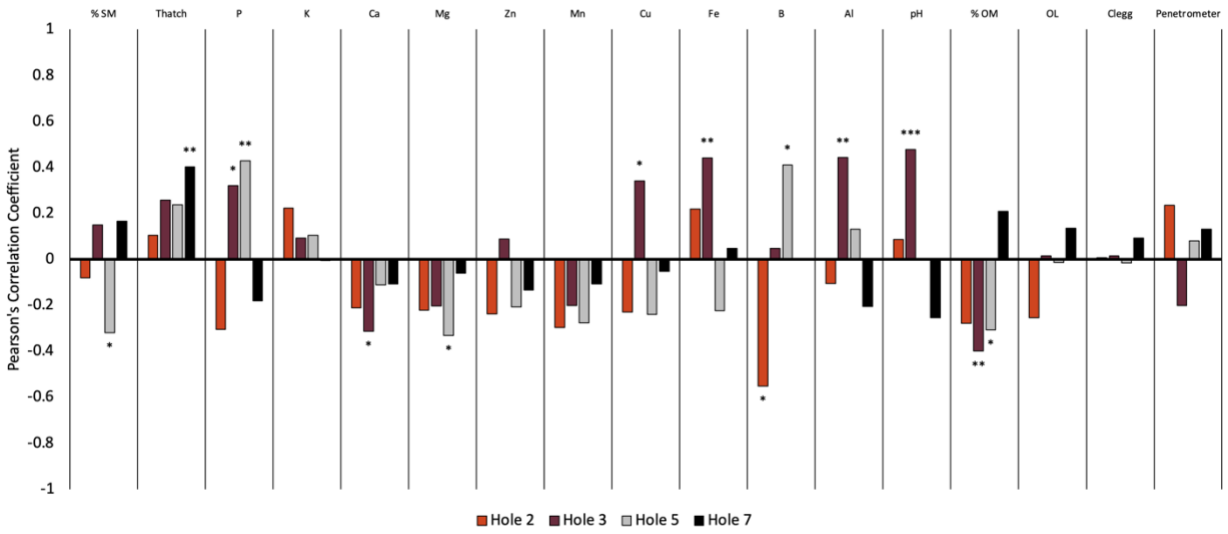

Figure 5. Pearson's correlation coefficients (PCC) and significance levels (* = P < 0.1; ** = P < 0.05; *** = P < 0.01) for percent spring dead spot and various environmental and edaphic factors (% SM = percent soil moisture; Thatch = thatch depth; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Zn = zinc; Mn = manganese; Cu = copper; Fe = iron; B = boron; Al = aluminum; pH = soil pH; % OM = percent organic matter in soil; OL = organic layer depth; Clegg = surface hardness measured with a Clegg soil impact tester; Penetrometer = surface hardness measured with a turf firmness meter) for 2021.

Table 1. Significant variables, their parameter estimates (slopes), and the model prediction expression were determined using a best subset stepwise regression analysis. The data presented in this table are for the spring dead spot patch numbers in 2020 for each hole sampled.

Hole #	Significant Variables	Estimate (Slope)	P-Value	Model Prediction Expression
2	pH	14.1220	0.0037	y = -63.4482 – 0.3902(OL) + 14.1220(pH)
	Organic Layer	-0.1895	0.0643	
3	Boron	-30.3907	<0.0001	y = -166.7389 – 30.3907(B) + 33.0516(pH)
	pH	33.0516	0.0033	
5	Organic Layer	-0.2335	0.0009	y = 9.7540 – 0.2335(OL) + 1.0132(P)
	Phosphorus	1.0132	0.0365	
7	Boron	20.9082	<0.0001	y = -0.4746 – 0.0912(Mg) + 20.9082(B)
	Magnesium	-0.0912	0.0001	

Table 2. Significant variables, their parameter estimates (slopes), and the model prediction expression were determined using a best subset stepwise regression analysis. The data presented in this table are for the spring dead spot patch numbers in 2021 for each hole sampled.

Hole #	Significant Variables	Estimate (Slope)	P-Value	Model Prediction Expression
2	Calcium	0.0481	<0.0001	y = -2.1417 + 0.0481(Ca) – 2.6218(Mn) – 0.0977(Fe) – 12.6571(B) + 0.1920(Al)
	Manganese	-2.6218	<0.0001	
	Aluminum	0.1920	<0.0001	
	Iron	-0.0977	<0.0001	
	Boron	-12.6571	<0.0001	
3	Magnesium	-0.3191	<0.0001	y = 4.0706 + 0.3778(K) – 0.3191(Mg) – 1.0447(Cu)
	Potassium	0.3778	0.0029	
	Copper	-1.0447	0.0039	
5	Thatch	0.4999	<0.0001	y = -18.1359 + 0.4999(Thatch) + 0.7076(P) – 0.9943(Cu)
	Copper	-0.9943	<0.0001	
	Phosphorus	0.7076	0.0003	
7	% Organic Matter	7.8037	<0.0001	y = -15.3479 + 0.2286 (% SM) + 0.0174(Penetrometer) – 36.3667(B) + 7.8037(% OM)
	Boron	-36.3667	<0.0001	
	Penetrometer	0.0174	0.0006	
	% Soil Moisture	0.2286	0.0125	

Table 3. Significant variables, their parameter estimates (slopes), and the model prediction expression were determined using a best subset stepwise regression analysis. The data presented in this table are for the percent spring dead spot in 2020 for each hole sampled. No significant variables were determined for Hole 2 and Hole 5. These two holes are denoted with N/A.

Hole #	Significant Variables	Estimate (Slope)	P-Value	Model Prediction Expression
2	N/A	N/A	N/A	N/A
3	Boron	-53.2971	<0.0001	y = 16.9306 + 0.5592(Thatch) – 53.2971(B) –4.4476(% OM)
	% Organic Matter	-4.4476	0.0035	
	Thatch	0.5592	0.0045	
5	N/A	N/A	N/A	N/A
7	Aluminum	-0.1935	0.0020	y = -124.2720 + 0.3265(K) – 0.1434(Mg) – 0.1935(Al) + 26.5302(pH)
	Potassium	0.3265	0.0047	
	pH	26.5302	0.0105	
	Magnesium	-0.1434	0.0267	

Table 4. Significant variables, their parameter estimates (slopes), and the model prediction expression were determined using a best subset stepwise regression analysis. The data presented in this table are for the percent spring dead spot in 2021 for each hole sampled.

Hole Number	Significant Variables	Estimate (Slope)	P-Value	Model Prediction Expression
2	Manganese	-2.0751	0.0005	y = -12.5169 + 0.9327(Thatch) – 2.0751(Mn)
	Thatch	0.9327	0.0035	
3	Potassium	0.8270	<0.0001	y = -259.3006 + 0.3849(% SM) + 0.2273(Clegg) + 0.0931(Penetrometer) + 0.8270(K) – 0.4610(Mg) – 1.7695(Cu) + 36.4532(pH)
	Penetrometer	0.0931	<0.0001	
	Magnesium	-0.4610	<0.0001	
	Copper	-1.7695	<0.0001	
	pH	36.4532	<0.0001	
	Clegg	0.2273	<0.0001	
	% Soil Moisture	0.3849	<0.0001	
5	Phosphorus	1.1903	<0.0001	y = -15.9373 + 0.0375(Penetrometer) + 1.1903(P)
	Penetrometer	0.0375	0.0199	
7	Thatch	0.2548	0.0009	y = -10.9262 + 0.2548(Thatch) – 0.1209(Mg) + 4.9431(% OM)
	Magnesium	-0.1209	0.0023	
	% Organic Matter	4.9431	0.0025	

Chapter 7: Cultivation and Fertility Practices Influence Hybrid Bermudagrass Recovery from Spring Dead Spot Damage

Abstract

Spring dead spot (SDS), caused by *Ophiosphaerella* spp., is among the most damaging diseases to hybrid bermudagrass (*Cynodon dactylon* x *transvaalensis*) in areas where winter dormancy occurs. Management strategies that aid in turfgrass recovery from SDS damage have not been widely studied. An experiment was conducted in Blacksburg, VA, USA in 2019 and 2020 to determine the influence of various cultural practices on bermudagrass recovery from SDS damage. Fertility and cultivation were applied in the late spring/early summer, which is earlier than normal for cultivation practices on bermudagrass, to test their effectiveness in aiding bermudagrass recovery from SDS damage. The main effects of fertility and cultivation were arranged in a 2 x 3 factorial design with vertical mowing, solid-tine aerification, and no cultivation applied with urea (48.8 kg N ha^{-1}) sprayed at trial initiation and two weeks later or without urea. Plots were assessed for percent SDS throughout the study. Data were analyzed as percent change relative to initial assessment in order to measure bermudagrass recovery. The main effect of fertility increased bermudagrass recovery from SDS damage in both 2019 and 2020. The main effects of vertical mowing and solid-tine aerification reduced bermudagrass recovery from SDS damage in 2020. These data suggest that two properly timed N fertilization applications at 48.8 kg ha^{-1} optimized bermudagrass recovery from SDS damage while late spring/early summer cultivation without fertility may inhibit bermudagrass recovery.

Introduction

Spring dead spot (SDS), caused primarily by *Ophiosphaerella herpotricha* J. Walker, *O. korrae* (J. Walker & A.M. Smith) Shoemaker & C.E. Babcock (=*Leptosphaeria korrae* J. Walker & A.M. Smith), and *O. narmari* (J. Walker & A.M. Smith) Wetzel, Hulbert, & Tisserat (=*L. narmari* J. Walker & A.M. Smith) in the U.S., is a common and detrimental patch disease of hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *transvaalensis* Burtt Davy) home lawns, athletic fields, and golf courses in areas where winter dormancy occurs. Patches appear sunken, necrotic, and straw-colored (Wadsworth and Young, 1960). The sunken nature of the patches decreases not only the aesthetics, but also the surface playability and player safety of hybrid bermudagrass for golf and sport uses (Martin et al., 2001). Many preventative chemical and cultural practices are employed to mitigate this disease.

Fungicide treatment is one of the primary management strategies for SDS prevention. Fungicides targeted at SDS are typically applied in the fall when soil temperatures are between 15.5 and 26.7°C (Butler and Tredway, 2006). Fungicide efficacy against SDS has been sporadic and efforts to increase fungicide efficacy through various application methods have produced mixed results (Beck et al., 2012; Butler and Tredway, 2006; Earlywine and Miller, 2015; Tredway et al., 2009b; Walker, 2013). New chemistries, particularly within the demethylase inhibitor (DMI) and succinate dehydrogenase inhibitor (SDHI) fungicide classes, are effective at inhibiting *Ophiosphaerella* spp. growth (Hutchens et al., 2019). However, cost of these newer fungicides may be a limiting factor for some turfgrass managers.

Cultural practices are frequently employed by turfgrass managers to prevent SDS, and an abundance of research has been conducted with mixed results (Tredway et al., 2009b). Miller et al. (2017) showed that nitrogen and manganese source had no effect on SDS severity the following

year while other studies demonstrated that nitrogen source differentially affected *O. herpotricha* and *O. korrae* (Tredway et al., 2009a; Tredway et al., 2020). Dernoeden et al. (1991) found that potassium chloride can increase survivability of bermudagrass inoculated with *O. korrae*; however, McCarty et al. (1992) determined that potassium sulfate actually increased SDS severity. Moreover, lowering pH with fertilizers such as ammonium sulfate has been shown to reduce SDS symptoms in the field and suppress mycelial growth *in vitro* (Cottrill et al., 2016; Dernoeden et al., 1991; Tredway et al., 2020). Furthermore, there is documented evidence of a positive correlation between pH and SDS severity, particularly with *O. herpotricha* (Dernoeden et al., 1991; Tredway et al., 2020). Cold tolerance of bermudagrass cultivars positively correlate with reduced SDS in certain studies, yet cold tolerant cultivars are not entirely immune from damage (Baird et al., 1998; Iriarte et al., 2005ab; Martinez et al., 2014). Cultivation practices that remove thatch such as fraze mowing, sod stripping, and aerification + vertical mowing during the summer can reduce SDS development the following spring (Miller et al., 2017; Tisserat and Fry, 1997). In contrast, vertical mowing or core aerifying was also demonstrated to increase SDS severity the following spring compared to a nontreated control (Perry et al., 2010). The results are unclear on the effect of cultivation practices on SDS prevention.

Research on curative and recuperative management strategies for symptomatic bermudagrass is limited. There have been studies focusing on which fertilizers most effectively increase bermudagrass recovery from SDS. Dernoeden et al. (1991) found that ammonium-based fertilizers generally provided the greatest SDS recovery. The authors reported on one rating date at the Denton, MD location that a fertilizer treatment of $(NH_4)_2SO_4$ provided greater bermudagrass recovery from SDS damage than $NaNO_3$, NH_4Cl, $CO(NH_2)_2$, $NaNO_3$ + KCl, $CO(NH_2)_2$ + KCl, and no fertility with the $NaNO_3$ + KCl treatment providing the least recovery. However, the authors

reported conflicting results at the Silver Spring, MD location with all fertility treatments similarly increasing bermudagrass recovery compared to the nontreated control with the exception of KCl. Ascocarps of *O. korrae* were observed at the Silver Spring location while no attempt of identification of the *Ophiosphaerella* species at the Denton location was made (Dernoeden et al., 1991). Polymerase chain reaction (PCR) methods for identifying *Ophiosphaerella* species were not available during the time of the Dernoeden et al. (1991) study, so identification of the species was more difficult. Recent data from our lab gathered from using species-specific primers developed by Tisserat et al. (1994) and Martinez et al. (2019) suggests that *O. korrae* is predominantly isolated from the Silver Spring area while *O. herpotricha* is predominant on the eastern shore of Maryland where Denton is located with 62% of the 42 samples collected from the eastern shore of Maryland amplifying *O. herpotricha* (Hutchens et al., 2021). Potential differences in the *Ophiosphaerella* species may be the cause of the discrepancies in bermudagrass recovery between the two locations in this study.

There has been research on the effects of preventative applications of fertility and cultivation on SDS caused by *O. herpotricha*, but there is no documented research on the influence of therapeutic cultivation and fertility applications on bermudagrass recovery, during the growing season, from SDS caused by *O. herpotricha* (Dernoeden et al., 1991; Miller et al., 2017; Tisserat and Fry, 1997; Tredway et al., 2009a; Tredway et al., 2020). Most research efforts addressing the influence of cultivation practices on SDS have been aimed at prevention of SDS the following spring (Miller et al., 2017; Tisserat and Fry, 1997). Based on review of the literature, there have been no studies on how cultivation practices such as vertical mowing and solid-tine aerification affect SDS recovery within the same growing season. Moreover, SDS patches can be slow to recover due to either the use of certain preemergence herbicides or metabolites produced by the

pathogen that can inhibit shoot regrowth (Beck et al., 2013; Fermanian et al., 1981; Venkatasubbaiah et al., 1994). Therefore, methods to aid in bermudagrass recovery from SDS damage are needed. The objective of our studies was to determine how implementation of late spring/early summer vertical mowing, solid-tine aerification, and urea (46-0-0) applications influence bermudagrass recovery from SDS within the same growing season.

Materials and Methods

Site Description

A field study was conducted on SDS symptomatic 'Latitude 36' hybrid bermudagrass at the Virginia Tech Practice Football Field (VTPFF), Blacksburg, VA, USA and SDS symptomatic 'NorthBridge' hybrid bermudagrass at Blacksburg Country Club (BCC), Blacksburg, VA, USA growing in urban soils. Based on a recently conducted *Ophiosphaerella* species geographic distribution study, *O. herpotricha* is primarily isolated in the Blacksburg area where both of our study sites were located (Hutchens et al., 2021). Studies were conducted at the VTPFF from 24 May 2019 to 2 Aug 2019 and repeated the following year from 25 Jun 2020 to 6 Aug 2020. Studies at BCC were conducted from 30 May 2019 to 12 Aug 2019 and repeated the following year from 25 Jun 2020 to 6 Aug 2020. The Latitude 36 hybrid bermudagrass at the VTPFF was maintained at 1.3 cm with an irrigation regiment sufficient to maintain turf vigor and prevent wilt while the NorthBridge hybrid bermudagrass at BCC was maintained at 1.3 cm with no supplemental irrigation. No preventative SDS fungicide applications were made at either study site.

Study Design and Treatments

In both studies the main effects of fertility and cultivation were arranged in a 2 x 3 factorial design in complete blocks with vertical mowing at a 1-cm depth from the soil surface, solid-tine aerification with 16-mm diameter tines at a 5.7-cm depth, and no cultivation applied with urea

(48.8 kg N ha^{-1}) sprayed at trial initiation and two weeks later or without urea. There were four replications, and treatments were applied to 1.8 m x 1.8 m plots with a CO_2-pressurized sprayer delivering solution at 276 kPa of pressure at a carrier volume of 842 L ha^{-1}. Urea treatments were irrigated within 1 hr after application in an attempt to prevent severe foliar burn from the fertilizer. Individual treatments are listed in Table 1. The same plots for each treatment were used in both study years.

Data Collection and Analysis

Plots were visually assessed every one to two weeks throughout the duration of the study for percent SDS. Percent SDS was measured as the percent area of the plot expressing sunken and/or necrotic patches with at least a 7.6-cm diameter area of necrosis. All data were transformed to percent change relative to initial assessment within plot. The additive inverse of the percent change relative to initial assessment is equal to the percent bermudagrass recovery. The equation is as follows, where *n* is equal to any given assessment date: Percent Recovery = (($Assessment_{Initial}$ - $Assessment_{n}$) / $Assessment_{Initial}$) x 100. Initial assessments were made on the day of study initiation. The range of initial percent SDS within each plot at each location was as follows: VTPFF in 2019 (1-18%), VTPFF in 2020 (0.6-20%), BCC in 2019 (2-35%), and BCC in 2020 (1.5-20%). Final assessments were made on 2 Aug 2019 and 6 Aug 2020 at VTPFF and on 12 Aug 2019 and 6 Aug 2020 at BCC. Relative percent SDS compared to initial SDS (i.e., percent recovery) was converted to area under the disease progress curve (AUDPC) to encompass the factor of time and bermudagrass recovery from SDS damage. The main effects were separated by year for each location. Data were subjected to ANOVA and means were separated using a Student's *t*-test. Means were separated with a significance level of $P \leq 0.1$ in JMP Pro 15 (SAS Institute, Cary, NC).

Results

The main effect of fertility increased bermudagrass recovery in both 2019 and 2020 ($P \leq 0.0417$) at BCC (Table 2). In 2019 fertility increased bermudagrass recovery by 19% compared to non-fertilized plots (Fig. 1). Similarly, in 2020, fertility increased bermudagrass recovery by 33% compared to non-fertilized plots (Fig. 1). The main effect of fertility influenced bermudagrass recovery ($P = 0.0629$) at VTPFF in 2019, but fertility did not influence bermudagrass recovery in 2020 ($P = 0.4907$) (Table 3). Fertility increased bermudagrass recovery by 40% in 2019 at VTPFF (Fig. 2).

Although there were negative trends on bermudagrass recovery in both years at BCC, cultivation practice did not have a significant effect on bermudagrass recovery in 2019 ($P = 0.2023$), but it did negatively impact bermudagrass recovery in 2020 ($P = 0.0071$) (Table 2). Bermudagrass recovery for solid-tine aerification and vertical mowing were not significantly different, and both cultivation practices reduced bermudagrass recovery by > 32% compared to no cultivation (Fig. 3). There was no significant main effect of cultivation at VTPFF in 2019 ($P = 0.6420$) or 2020 ($P = 0.5304$) (Table 3), but the trends were similar to BCC with solid-tine aerification and vertical mowing numerically reducing bermudagrass recovery. There were no fertility*cultivation interaction effects at BCC or VTPFF.

Discussion

Our data suggest that late spring/early summer nitrogen applications are beneficial to turf recovery from SDS damage. Dernoeden et al. (1991) had similar findings to our studies with spring/summer applications of nitrogen increasing bermudagrass recovery from SDS damage. The authors showed that all sources of nitrogen evaluated enhanced turf recovery from SDS damage, yet the rate of recovery compared to a nontreated control differed among nitrogen sources. We

found that late spring/early summer applications of urea were beneficial for turf recovery from SDS damage suggesting that nitrogen is the key nutrient for turf recovery. Similarly, Dernoeden et al. (1991) showed that urea increased bermudagrass recovery from SDS. This has been documented for dead spot of creeping bentgrass (*Ophiosphaerella agrostis*) as well (Kaminski and Dernoeden, 2005).

We observed that plots treated with fertility in 2019 had less initial SDS in 2020 relative to initial SDS in 2019 ($P = 0.0718$) at VTPFF (data not shown). This provides further support that nitrogen applications could be beneficial for SDS suppression; however, no significant reduction of SDS in 2020 from fertility applications in 2019 at BCC were observed (data not shown). Ammonium sulfate and calcium nitrate applications have also been shown to reduce SDS damage depending on which *Ophiosphaerella* species is present (Dernoeden et al., 1991; Tredway et al., 2020). Our results are different from what McCarty et al. (1992) reported with sulfur-coated urea increasing SDS up to 128%. Nitrogen applications in our study were made in late spring/early summer while nitrogen applications in the McCarty et al. (1992) study were made in the fall offering a potential reason for the different results. Moreover, the addition of sulfur to the plant from the use of sulfur-coated urea by McCarty et al. (1992) may have been the reason for the increased SDS, which has also been documented by Perry et al. (2010).

Urea is a cost-effective fertilizer, but it only provides nitrogen to the plant and misapplications can cause phytotoxicity due to its high water solubility (Krogmeier et al., 1989). Less soluble fertilizers delivering nitrogen, as well as other required macro- or micro-nutrients, may be better options than urea to apply on bermudagrass in the late spring/early summer to enhance overall bermudagrass health as well as recovery from SDS damage. Further research

needs to be conducted on the effect of nitrogen source, other nutrients, and application timing on bermudagrass recovery and prevention from SDS damage.

Our study showed that cultivation was inhibitory to bermudagrass recovery at BCC but not VTPFF. The VTPFF location was more intensively managed than the BCC location, which may have reduced the inhibitory effects of cultivation to bermudagrass recovery from SDS. No research has been published on the influence of cultivation practices on SDS recovery within the same season, but research on the use of cultivation practices for prevention of SDS is well documented. Tisserat and Fry (1997) showed that vertical mowing or aerifying, alone, did not reduce SDS symptom expression the following spring, which is similar to what we observed (data not shown). However, they demonstrated that the combination of vertical mowing and aerifying did reduce SDS symptom expression the following spring. Miller et al. (2017) showed similar results from treatments employing the highly disruptive fraze mower. In contrast, Perry et al. (2010) demonstrated that core aerifying or vertical mowing in the summer can increase SDS the following spring. However, the effect of increased SDS with cultivation practices was only observed in one year of a three-year study (Perry et al., 2010). The Tisserat and Fry (1997) and Miller et al. (2017) studies suggest that highly disruptive cultivation practices are beneficial for preventing SDS the following season, which is likely due to thatch reduction and removal, increased oxygen to the rootzone, and generation of new growing points on the plant. Our data suggest that early season cultivation, particularly in the absence of fertility, can decrease bermudagrass recovery from SDS within the same season. Although disruptive cultivation practices can be beneficial for prevention of SDS, they do not aid bermudagrass in recovery from SDS damage within the same season, particularly in the absence of nitrogen fertility.

Our data indicate that up to 97.6 kg N ha^{-1} from urea applied over a two-week period in late spring/early summer greatly accelerated bermudagrass recovery from SDS. Cultivation practices should be implemented after bermudagrass has mostly recovered from SDS damage, which is often mid-summer. If 97.6 kg N ha^{-1} is applied in late spring and cultivation conducted in mid-summer, bermudagrass recovery from SDS damage should be optimized. However, more research needs to be conducted to determine the effect of various nitrogen sources and application timings on bermudagrass recovery from SDS damage.

References

Baird, J. H., D.L. Martin, C.M. Taliaferro, M.E. Payton, and N.A. Tisserat. 1998. Bermuda- grass resistance to spring dead spot caused by *Ophiosphaerella herpotricha.* Plant Dis. 82:771-774. https://doi.org/10.1094/PDIS.1998.82.7.771

Beck, L.L., T. Cooper, A.J. Hephner, C.M. Straw, and G.M. Henry. 2013. Effect of preemergence herbicides on the recovery of bermudagrass from spring dead spot. Appl. Turfgrass Sci. 10. https://doi.org/10.1094/ATS-2013-0328-01-RS

Beck, L.L., J. Moore-Kucera, G. Henry, J. Woodward, J. Zak, and R. Cox. 2012. Evaluation of chemical and cultural methods for the management of spring dead spot in bermudagrass Turf. Dissertation. Texas Tech Univ., Lubbock, TX. Retrieved from https://ttu-ir.tdl.org/bitstream/handle/2346/50750/Beck_Leslie_Diss.pdf?sequence=1&isAllowed=y

Butler, E.L. and L.P. Tredway. 2006. Method and timing of fungicide applications for control of spring dead spot in hybrid bermudagrass. Online. Plant Health Prog. https://doi.org/10.1094/PHP-2006-0901-01-RS

Cottrill, D.J., D.T. Earlywine, and G.L. Miller. 2016. Assessment of nitrogen source, sulfur, and fall fungicide applications on the management of spring dead spot of bermudagrass. Plant Dis. 100:473-482. https://doi.org/10.1094/PDIS-05-15-0565-RE

Dernoeden, P.H., J.N. Crahay, and D.B. Davis. 1991. Spring dead spot and bermudagrass quality as influenced by nitrogen source and potassium. Crop Sci. 31:1674-1680. https://doi.org/10.2135/cropsci1991.0011183X003100060058x

Earlywine, D.T. and G.L. Miller. 2015. Evaluation of multiple fungicides in combination with a wetting agent for spring dead spot control on bermudagrass, 2013-2014. Plant Dis. Manag. Rep. 9:T018.

Fermanian, T.W., R.M. Ahring, and W.W. Huffine. 1981. The isolation of a toxin from spring dead spot areas in bermudagrass (Cynodon L.C. Rich) turf. Intl. Turfgrass Soc. Res. J. 4:433-441.

Hutchens, W.J., C.A. Henderson, E.A. Bush, J.P. Kerns, and D.S. McCall. 2021. Geographic Distribution of *Ophiosphaerella* Species in the Mid-Atlantic United States. *Plant Health Prog*. https://doi.org/10.1094/PHP-04-21-0076-S.

Hutchens, W.J., Y. Nagaoka, J.P. Kerns, J.M. Goatley, M. Nita, and D.S. McCall. 2019. Variable sensitivity of *Ophiosphaerella* spp. causing spring dead spot to fungicides and temperature. Abstract. *Crop Science Society of America* Annual Meeting, 2019.

Iriarte, F.B., H.C. Wetzel III, J.D. Fry, D.L. Martin, P. Vincelli, E.W. Dixon, and N.A. Tisserat. 2005a. Aggressiveness of spring dead spot pathogens to bermudagrass. Int. Turf. Res. J. 10:258-264.

Iriarte, F.B., J.D. Fry, D.L. Martin, T.C. Todd, and N.A. Tisserat. 2005b. Effect of cold acclimation and freezing on spring dead spot severity in bermudagrass. HortScience 40:421-423. https://doi.org/10.21273/HORTSCI.40.2.421

Kaminski, J.E., and P.H. Dernoeden. 2005. Nitrogen source impact on dead spot (*Ophiosphaerella agrostis*) recovery in creeping bentgrass. Int. Turfgrass Soc. Res. J. 10:214-223.

Krogmeier, M.J., G.W. McCarty, and J.M. Bremner. 1989. Phytotoxicity of foliar-applied urea. Proc. Natl. Acad. Sci. 86:8189-8191. https://doi.org/10.1073/pnas.86.21.8189

Martin, D.L, C.M. Taliaferro, N.A. Tisserat, G.E. Bell, J.H. Baird, D.D. Dobson, J.A. Anderson, and R.M. Kuzmic. 2001. Hardy bermudagrass sought with resistance to spring dead spot. *Golf Course Management* June 2001:75-79.

Martinez, J.F.I., F.J. Flores, A.R. Koch, C.D. Garzon, and N.R. Walker. 2019. Multiplex end-point PCR for the detection of three species of *Ophiosphaerella* causing spring dead spot of bermudagrass. Plant Dis. 103:2010-2014. https.//doi.org/10.1094/PDIS-10-18-1727-RE

Martinez, A., J.B. Workman, and F.C. Waltz. 2014. Identification and control of spring dead spot in Georgia. UGA Extension Circular 1012.

McCarty, L.B., L.T. Lucas, and J.M. DiPaola. 1992. Spring dead spot occurrence in bermudagrass following fungicide and nutrient applications. HortSci. 27.10:1092-1093. https://doi.org/10.21273/HORTSCI/27.10.1092

Miller, G.L., D.T. Earlywine, and B.S. Fresenburg. 2017. Effect of fraze mowing on spring dead spot caused by *Ophiosphaerella herpotricha* of bermudagrass. Int. Turfgrass Soc. Res. J. 13:225-228. https://doi.org/10.2134/itsrj2016.10.0839

Perry, D.H., M. Tomaso-Peterson, and R. Baird. 2010. Seasonal variation in frequency of isolation of *Ophiosphaerella korrae* from bermudagrass roots in Mississippi and pathogenicity and optimal growth of selected isolates. Mycopathologia 169:395-402. https://doi.org/10.1007/s11046-010-9273-x

Tisserat, N.A. and J.D. Fry. 1997. Cultural practices to reduce spring dead spot (*Ophiosphaerella herpotricha*) severity in *Cynodon dactylon*. J. Intl. Turf. Res. 8:931-936.

Tisserat, N.A., S.H. Hulbert, and K.M. Sauer. 1994. Selective Amplification of rDNA Internal Transcribed Spacer Regions to Detect *Ophiosphaerella korrae* and *O. herpotricha*. Phytopathology 84:478-482. https://doi.org/10.1094/phyto-....

Tredway, L.P., M.D. Soika, and E.L. Butler. 2009a. Response of spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha* to fertilization programs and preventative fungicide applications. Phytopathology 99.6:S129

Tredway, L.P., M.D. Soika, E.L. Butler, and J.P. Kerns. 2020. Impact of nitrogen source, fall fertilizers, and preventative fungicides on spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha*. Crop Sci. Special Issue: International Turfgrass Research Conference: 1-10. https://doi.org/10.1002/csc2.20306

Tredway, L. P., M. Tomaso-Peterson, H. Perry, and N.R. Walker. 2009b. Spring dead spot of bermudagrass: A challenge for researchers and turfgrass managers. Online. Plant Health Progress https://doi.org/10.1094/PHP-2009-0710-01-RV

Venkatasubbaiah, P., N.A. Tisserat, and W.S. Chilton. 1994. Metabolites of *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermudagrass. Mycopathologia 128:155-159. https://doi.org/10.1007/BF01138477.

Wadsworth, D. F. and H.C. Young. 1960. Spring dead spot of bermudagrass. Plant Dis. 44:516-518.

Walker, N.R. 2013. Evaluation of post fungicide application irrigation on control of spring dead spot of bermudagrass in Oklahoma, 2012-2013. Plant Dis. Manag. Rep. 8:T011.

Table 1. Treatments for spring dead spot recovery trials at Blacksburg Country Club and the Virginia Tech Practice Football Field.

Cultivation[1]	Fertility[2]	Treatment Name
None	None	Nontreated control
Vertical mowing	None	Vertical mowing
Solid-tine aerification	None	Solid-tine aerification
None	96.7 kg N ha^{-1}	Urea
Vertical mowing	96.7 kg N ha^{-1}	Urea + vertical mowing
Solid-tine aerification	96.7 kg N ha^{-1}	Urea + solid-tine aerification

[1]Vertical mowing was applied at trial initiation at a 1-cm depth and solid-tine aerification was applied at a 5.7-cm depth at trial initiation with 16-mm diameter tines.

[2]All fertility applications were made with urea at a rate of 48.8 kg N ha^{-1} applied at trial initiation and two weeks later.

Table 2. Analysis of variance for main effects of fertilization, main effects of cultivation, and interaction effects on bermudagrass recovery from spring dead spot at Blacksburg Country Club in 2019 and 2020.

Year	Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
2019	Cultivation	2	2439014	1219507	1.7810	0.2023
	Fertility	1	3396236	3396236	4.9599	0.0417
	Cultivation*Fertility	2	2567146	1283573	1.8745	0.1876
	Block	3	11523729	3841243	5.6098	0.0088
	Error	15	10271151	684743		
	Total	23	30197275			
2020	Cultivation	2	8874224.4	4437112.2	7.0148	0.0071
	Fertility	1	3453958.5	3453958.5	5.4605	0.0337
	Cultivation*Fertility	2	1615208.4	807604.2	1.2768	0.3076
	Block	3	1775241.1	591747.0333	0.9355	0.4479
	Error	15	9487992	632533		
	Total	23	25206624			

Table 3. Analysis of variance for main effects of fertilization, main effects of cultivation, and interaction effects on bermudagrass recovery from spring dead spot at Virginia Tech Practice Football Field in 2019 and 2020.

Year	Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
2019	Cultivation	2	724134	362067	0.4565	0.6420
	Fertility	1	3200442	3200442	4.0355	0.0629
	Cultivation*Fertility	2	2330997	1165498.5	1.4696	0.2613
	Block	3	14354870	4784956.7	6.0335	0.0066
	Error	15	11895949	793063		
	Total	23	32506393			
2020	Cultivation	2	1233570.3	616.785.15	0.6617	0.5304
	Fertility	1	464831.4	464831.4	0.4991	0.4907
	Cultivation*Fertility	2	831117.9	415558.95	0.4462	0.6483
	Block	3	3782162.8	1260720.93	1.3537	0.2948
	Error	15	13969938	788835		
	Total	23	20280620			

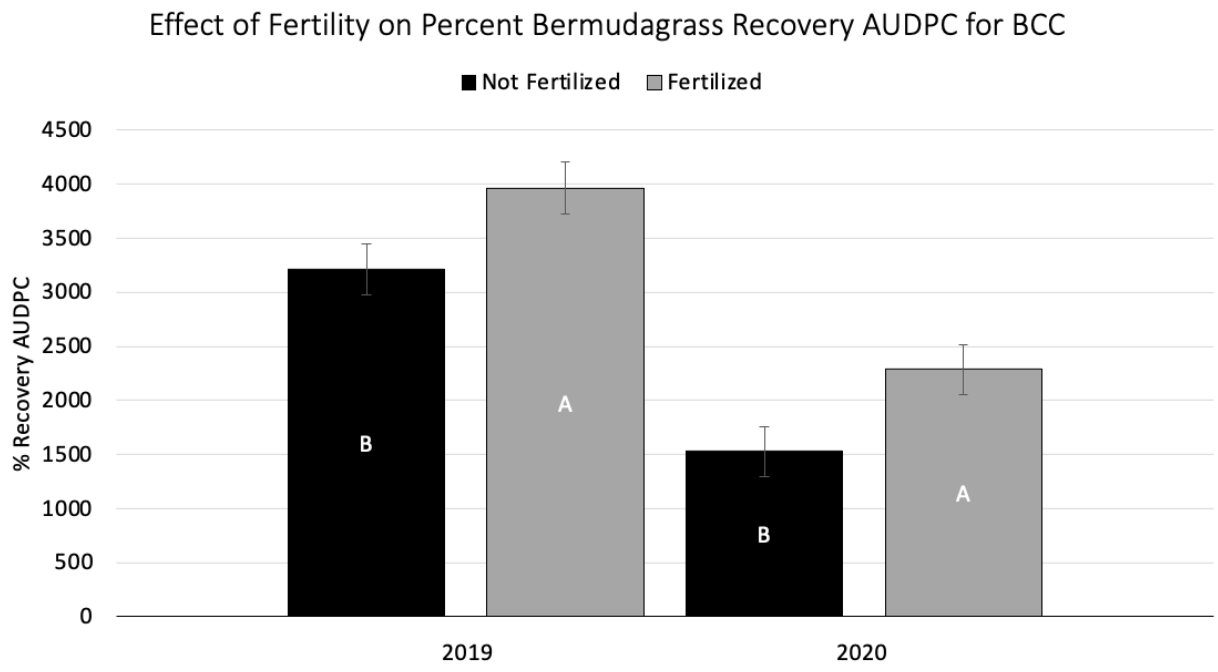

Figure 1. Main effect of fertility on percent bermudagrass recovery area under the disease progress curve (AUDPC) at Blacksburg Country Club in 2019 and 2020. Different color bars represent different fertility practices, means are compared within year, and bars with different letters are significantly different ($P < 0.05$).

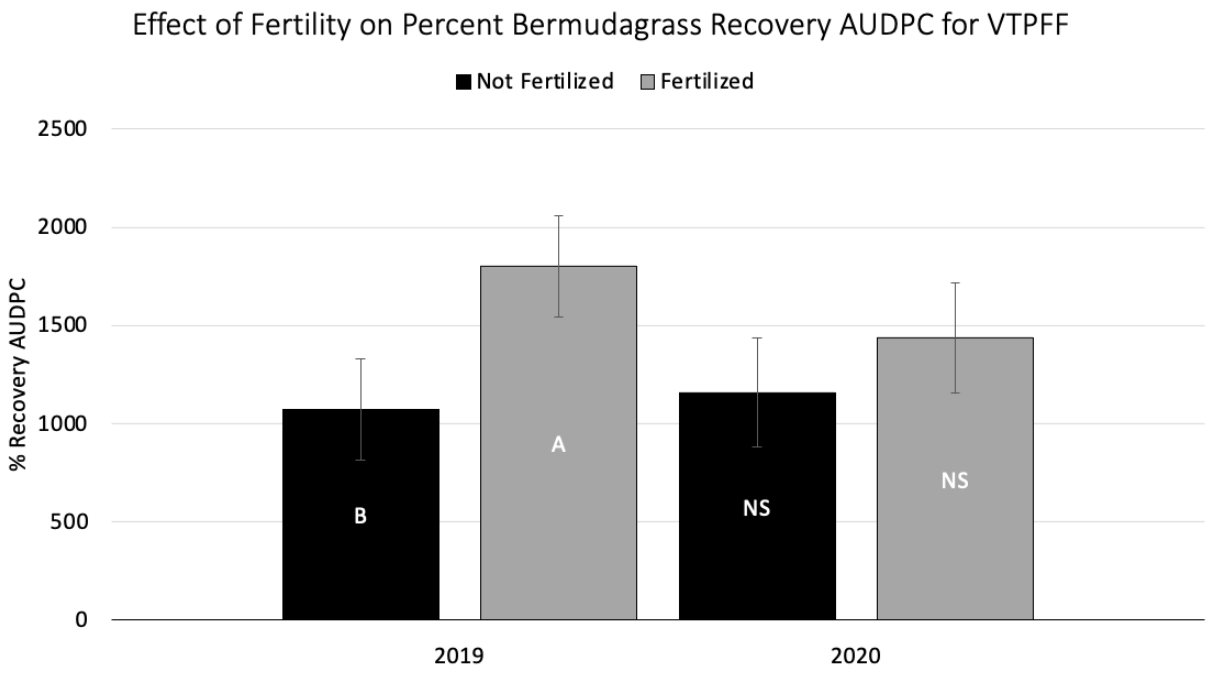

Figure 2. Main effect of fertility on percent bermudagrass recovery area under the disease progress curve (AUDPC) at Virginia Tech Practice Football Field in 2019 and 2020. Different color bars represent different fertility practices, means are compared within year, and bars with different letters are significantly different ($P < 0.1$).

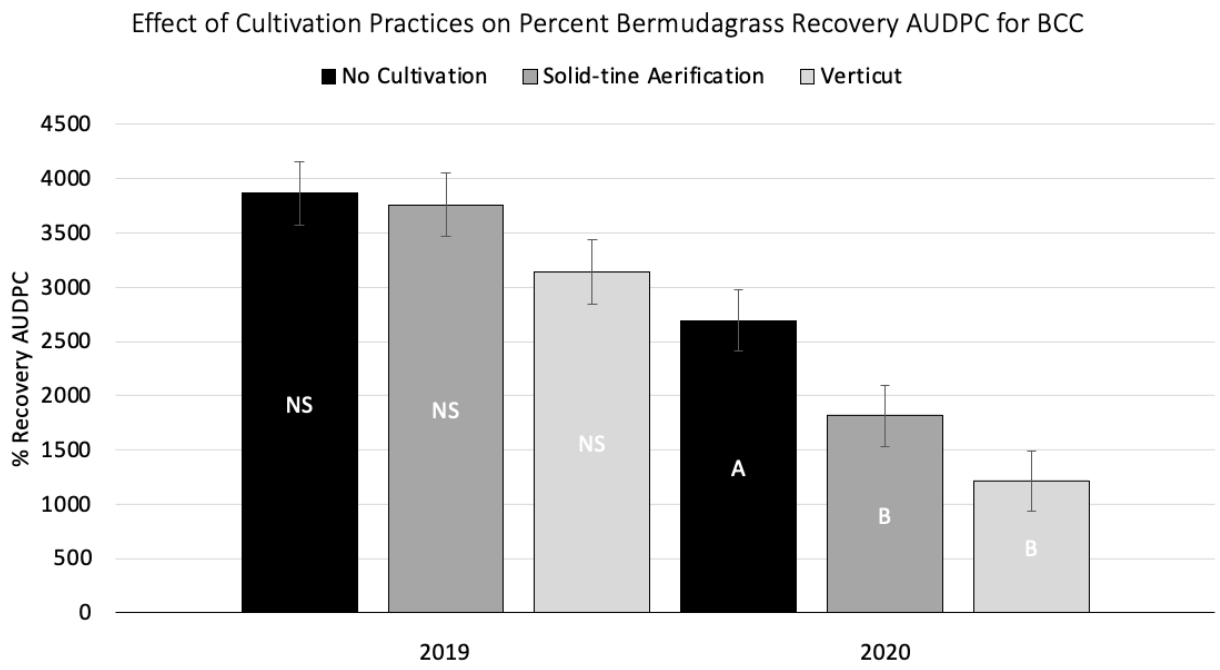

Figure 3. Main effect of cultivation practice on percent bermudagrass recovery area under the disease progress curve (AUDPC) at Blacksburg Country Club in 2019 and 2020. Different color bars represent different cultivation practices, means are compared within year, and bars with different letters are significantly different ($P < 0.05$).