ABSTRACT

FREUND, DANIEL ROBERT. Effect of Management Practices on Fungicide Efficacy and Environmental Fate in Turfgrass Systems (Under direction of Drs. Travis W. Gannon and James P. Kerns).

Understanding fungicide environmental fate and behavior enables turfgrass managers to maximize fungicide efficacy through use of best management practices. Researchers set out to investigate management practices that evaluated azoxystrobin, cyazofamid and ^{14}C mefenoxam persistence and distribution in turfgrass systems.

Field research was conducted to assess the effect of initial mowing timing on azoxystrobin-brown patch (*Rhizoctonia* spp.) efficacy in tall fescue *(Festuca arundinacea* Schreb.). Azoxystrobin was sprayed on unique plots and were mown at 0, 1, 2, 3, 7 or 14 days after treatment (DAT). Additionally, clipping management practices were evaluated following azoxystrobin application and rated for brown patch disease severity. To determine the effect of clipping management practices, unique plots were mown at 3, 10 and 17 DAT and clippings were either returned to the canopy or bagged and removed. Both experiments were visually rated weekly for brown patch (% severity) through 28 DAT. Azoxystrobin residue was quantified in tall fescue aboveground vegetation and below ground soil. Overall, researchers observed an increase in azoxystrobin concentration in the tall fescue vegetation when mowing timing was delayed. Returning clippings also increased the concentration of azoxystrobin residue in aboveground vegetation and soil compared to removing clippings when collected at 7 and 21 DAT. Brown patch severity (%) was not influenced by mowing timing or clipping management practice following an azoxystobin application, however increased residue may impact inoculum buildup.

Fungicides are necessary when managing root and crown diseases of turfgrasses. However, their physicochemical properties combined with the makeup of a USGA putting green does not allow for movement into the root zone. In order to facilitate fungicide movement, turfgrass managers use various practices, including soil surfactants and post fungicide application irrigation. Field and lab research were conducted to investigate best management practices to increase fungicide distribution in turf and soil profiles. Cyazofamid was applied and irrigated immediately or delayed 6 hours or 72 hours. Residue quantification revealed that immediate irrigation increased cyazofamid distribution through the soil profile into the rootzone. Greater concentration of cyazofamid was recovered in the verdure/thatch layer when irrigation was delayed, suggesting irrigation should be applied as soon as possible to maximize cyazofamid distribution. Lab research was conducted to evaluate vertical distribution of ^{14}C mefenoxam when applied with the soil surfactant Cascade Plus. ^{14}C-mefenoxam was applied to a replicated USGA soil lysimeter and was quantified within soil depths (0-2.5 cm) to 15 cm. Researchers observed enhanced movement of mefenoxam when soil surfactant was pre-treated 24 hours prior. Data indicate that immediate irrigation and soil surfactant inclusion may increase fungicide distribution in soil, potentially enhancing disease suppression.

Information from this research enhance our understanding of fungicide fate in turfgrass systems and helps growers establish best management practices to maximize efficacy by increasing fungicide delivery to targeted pathogens.

Effect of Management Practices on Fungicide Efficacy and Environmental Fate in Turfgrass Systems

by
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DEDICATION

I dedicate this thesis to every family member and friend of mine who has supported me on this journey over the past years.

BIOGRAPHY

Daniel R. Freund was born to Kevin and Carolyn Freund on October 28, 1994 in Raleigh , NC. He grew up in the Raleigh area, hunting, playing sports and spending time outside. By high school, he began working on his uncle's golf course where he developed an interest in turfgrass maintenance and agronomy. This interest led to him to pursue a B.S. degree in turfgrass science at North Carolina State University, completed in 2017. During his last semester, he began working as an undergraduate research assistant in Dr. Jim Kerns lab and began learning about fungicide research. He had the pleasure of teaming up with Dr. Travis Gannon and Dr. Jim Kerns and decided to pursue a M.S. degree at North Carolina State University in the Department of Crop and Soil Sciences, researching fungicide fate in turfgrass systems. He has been surrounded by great colleagues and advisors and gained Dr. Grady Miller on his advisory committee. Daniel began working as a graduate research technician with Dr. Gannon, expanding his research of pesticide fate in agronomic systems. Outside of work, Daniel likes to play golf and spend time with his Fiancé, family, friends and his dog Rhett.

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Previous research suggests mowing practices following azoxystrobin application alter pest control and residue fate. Field research was initiated June 14, 2018 in Raleigh, NC and repeated in time to assess the effect of post-application mowing timing and clipping management practices on azoxystrobin residue persistence in tall fescue (*Festuca arundinacea* Schreb.). During the experiments, brown patch (*Rhizoctonia* spp.) disease severity (%) ratings were taken. At trial initiation, azoxystrobin was applied at the maximum single application rate (0.61 kg ai ha^{-1}) to unique tall fescue plots with small-plot, CO_2-propelled equipment calibrated to deliver 812 L ha^{-1}. To determine the effect of initial mowing timing, plots were mown (9.5-cm) at 0, 1, 2, 3, 7 or 14 days after treatment (DAT). Regardless of mowing timing, clippings were mulched and returned to the canopy, and mown every 7 days following the first 7 DAT. To determine the effect of clipping removal, unique plots were mown at 3, 10 and 17 DAT and clippings were either returned to the canopy or bagged and removed. Both experiments were visually rated weekly for brown patch (% severity) through 28 DAT. Concurrently soil cores (92 cm^2) were collected at 3, 7, 14 and 21 DAT and then segmented into remaining aboveground vegetation and soil (0.0 to 2.5 cm depth) for subsequent residue analyses. Azoxystrobin residue in soil and vegetation was quantified via HPLC-DAD-MS methodology at the North Carolina State University Pesticide and Trace Elements Laboratory. At 14 DAT sampling time, immediate mowing presented a lower total recovery (19.4%) of applied azoxystrobin compared to mowing at 14 DAT (38.3%). When visually rated through 28 DAT, azoxystrobin provided excellent brown patch suppression (1.5 to 2.5%

diseased plot area) regardless of mowing timing compared to 13% brown patch in nontreated plots in 2018. When clippings were returned to the canopy, 5% more of applied azoxystobin was detected in the aboveground vegetation at 7 and 14 DAT. At 3, 7 and 21 DAT, in the soil matrix, returning clippings resulted in >3% more of the applied azoxystrobin compared to removing clippings. Brown patch disease pressure was higher in 2019, where the non-treated plots expressed higher disease severity at 21 and 28 DAT (21.7 and 26.6%, respectively). Data gathered from this research may allow turfgrass managers to extend azoxystrobin residue, which could be beneficial under increased disease pressure or may result in a reduction in overall fungicide inputs.

Abbreviations: DAT, days after treatment; MTAT, mowing timing after treatment

1 Introduction

Tall fescue (*Festuca arundinacea* Schreb.) was reported in 2004 as the most common turf species in North Carolina home lawns, grown on 37% (300,500 hectares) of the turf acreage (Brandenburg et al., 2004). Based on the most recent survey of turfgrass in North Carolina, turfgrass areas encompass 866,000 hectares, with 47.8% of the area planted with tall fescue (NC AG. Stats., 1999). North Carolina is in the transition zone, where tall fescue is able to grow in all three regions of the state. However, the piedmont and Appalachian Mountains regions are more conducive for growth, particularly through the summer. Tall fescue is selected for these regions due to its adaptability to multiple soil types, adequate shade and drought tolerance, ease of establishment, and relatively low fertility requirements. Despite the benefits of tall fescue, it is susceptible to diseases such as brown patch (*Rhizoctonia* spp.), Pythium blight (*Pythium* spp.) and gray leaf spot (*Pyricularia grisea* Sacc.). Warm and humid weather during summer months can be ideal for brown patch development and will commonly infect tall fescue. *Rhizoctonia* species are soilborne fungal pathogens that infect many cool- and warm-season turfgrass species (Turgeon, 1999). In tall fescue, brown patch is observed as large light brown, circular patches (~15-30 cm) containing sunken and depressed leaf tissue. Plant symptoms are distinctive in tall fescue, appearing often as tan or light brown lesions, with a darker brown or purple margin. (Smiley et al., 2005). The pathogens are able to survive and overwinter in thatch and turf debris between active periods.

Brown patch severity is lessened when cultural practices such as the use of resistant cultivars, proper irrigation timing and clipping management are employed. Yet, these may not be sufficient to minimize brown patch severity, requiring fungicides to reduce infection levels.

Fungicide management is an approach that turf managers will take in order to prevent or mitigate pathogen activity and infection on turfgrass plants. Azoxystrobin (methyl(E)-2-{2[6- (2-cyanophenoxy)pyrmidin-4-yloxy]phenyl}-3-methoxyacrylate) is a common fungicide used in turf and is effective against a wide spectrum of diseases caused by ascomycetes and basidiomycetes pathogens (Morton & Stuab, 2008; Young, 2020). Azoxystrobin is classified as a QoI fungicide and is able to interfere with energy production in fungal mitochondria, limiting energy production and inhibiting pathogen growth (Vincelli, 2002).

Established turfgrass canopies inherently intercept an appreciable portion of spray-applied pesticides. Previously, Jeffries et al. (2016) reported removing 30.1% of applied azoxystrobin from hybrid bermudagrass (*Cynodon dactylon* L. x *C. transvaalensis*), 20.0% from tall fescue and 17.2% from zoysiagrass (*Zoysia japonica* Steud.) when mown the same day as spray application. If fungicide residues are removed in turfgrass clippings, the fate of the clippings may influence disease incidence and severity. Lewis et al. (2014) reported that mown clippings collected from tall fescue treated with aminocyclopyrachlor (79 g ae ha^{-1}) 14 to 1.75 days before clipping collection reduced white clover 73 to 92% 8 weeks after clippings were applied to the stand, indicating that aminocyclopyrachlor is persistent and bioavailable in clippings of tall fescue previous treated with the herbicide. Researchers hypothesized that aminocyclopyrachlor present in the treated tall fescue clippings was released into the soil profile as the clipping decomposed, and absorbed by white clover roots, translocating into the foliage. More specific to fungicides, Frederick et al. (1994) reported >14% of the initial concentrations of chloroneb and triadimefon remained in Kentucky bluegrass (*Poa pratensis* L.) clippings for 56 days. Depending on the pesticide and system, evidence supports that pesticides in/on treated grass clippings may persist and be available for plant uptake.

Turfgrass systems are routinely mowed during periods of active growth, and often, it is recommended clippings be returned to the turfgrass canopy to recycle nutrients (Turgeon, 1999). Although recommended, this practice is not always observed as homeowners may bag their clippings. With some turfgrass systems (e.g., putting green), it may be necessary for clippings to be collected each time a mowing event occurs (Bruneau et al., 2008). Previous research has investigated the influence of returning clippings on the development of brown patch and Pythium blight in perennial ryegrass (*Lolium perenne* L.) and tall fescue (Shurtleff, 1953; Dunn et al., 1996; Settle & Fry, 2001). Shurtleff (1953) found that *R. solani* can survive up to 4 months on perennial ryegrass clippings. Dunn et al. (1996) evaluated the effect of N rate and clipping disposal on disease incidence and perennial ryegrass quality and observed increased brown patch severity when clippings were returned, suggesting recycling grass clippings may act as a source of inoculum for brown patch development. Authors determined that N is recycled to turf when clippings are returned, and N is removed when clippings are removed, suggesting higher N amounts may enhance brown patch incidence. Although these studies were conducted on perennial ryegrass, information can likely be extrapolated to a tall fescue turfgrass system. Settle and Fry (2001) reported brown patch incidence on tall fescue was not affected by collecting or returning clippings following azoxystrobin application; however, without residue quantification over time, it cannot be determined if mowing practices or sequential applications was the primary factor in brown patch control. Additionally, researchers conducted research in northwest Kansas, which generally experiences less severe conditions for brown patch incidence compare to transition regions of the southeast United States.

The use of fungicides to manage brown patch on tall fescue is effective whether applied preventatively or curatively. However, fungicide use is limited in some systems due to cost,

number of applications required, and difficulty in timing treatments (Tisserat and Pair, 1997). Once applied to a turfgrass canopy, fungicide persistence is influenced by a combination of abiotic and biotic factors. Some of these factors include removal of clippings, volatilization, photolysis, microbial degradation, breakdown in the plant, and runoff and leaching from rainfall events (Daniels & Latin, 2013). Degradation and dissipation processes likely influence disease suppression and a lack of understanding of these process may leave managers wondering why fungicide performance was impaired. Research completed in the present study was designed to evaluated management practices that may allow turfgrass managers to extend azoxystrobin persistence and achieve a longer period of efficacy. Two experiments were completed to characterize persistence and fate of azoxystrobin in a tall fescue system maintained as a home lawn. The first objective of this research was to evaluate the effect of mowing timing following azoxystrobin spray application on azoxystrobin efficacy. The second objective of this research was to evaluate the effect of clipping management practices following azoxystrobin spray application on efficacy. Azoxystrobin residue quantification allowed researchers to characterize fate within turfgrass components over time and simultaneously, brown patch severity ratings were taken. The combination of azoxystrobin residue quantification paired with disease severity ratings will help researchers better understand how management practices affect efficacy and enable development of best management practices to optimize azoxystrobin applications.

Core Ideas

- Limited research is available concerning mowing practices post-fungicide application and associated efficacy.
- Delayed mowing after azoxystrobin application did not affect brown patch control.

- Returned clippings increased azoxystrobin detection in vegetation and soil but did not affect brown patch control.

2 Materials and Methods

2.1 Research Overview

Two separate field experiments were conducted and quantified azoxystrobin residue in tall fescue aboveground vegetation (9.5 cm) and in soil (0-2.5 cm). The first experiment was conducted to evaluate the effect of initial mowing timing after azoxystrobin application on brown patch (*Rhizoctonia* spp.) control and azoxystrobin persistence in tall fescue. The second experiment was conducted to evaluate the effect of turfgrass clipping management practices after azoxystrobin application on brown patch control and azoxystrobin persistence in tall fescue. Both experiments were visually rated weekly for percent of plot area exhibiting symptoms (% severity), through 28 DAT. Research was conducted on a Cecil sandy loam soil with pH 5.8 and 1.9% w/w organic matter on an established 'Triple Threat' tall fescue stand. Azoxystrobin was not applied to research areas for 2 yr prior to initiation. Additionally, soil and vegetation samples from the areas were analyzed prior to initiation to confirm azoxystrobin residues were non-detectable. Turfgrass research areas were managed in accordance with recommendations with respect to fertility (100 kg N $ha^{-1}yr^{-1}$), irrigation (provided to supplement rainfall), and mowing height (9.5 cm) (Bruneau et al., 2008).

2.2 Influence of Mowing Timing

Research was initiated June 14, 2018 and repeated July 19, 2019 at Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC to investigate the influence of mowing timing following a

fungicide application. Three days prior to initiation, tall fescue was mown (9.5 cm height of cut), and clippings returned with a self-propelled rotary mower (Honda HRC 2163HXA®, American Honda Power Equipment Division, Alpharetta, GA). One day prior to trial initiation, the area was irrigated to field capacity. At initiation (11:00 AM EST), azoxystrobin (Heritage TL®; Syngenta Crop Protection, Inc., Greensboro, NC) was sprayed at the current single maximum application rate (0.61 kg ai ha^{-1}) to unique 1.5 by 3 m plots (0.9 m alleys between reps; 0.3 m alleys between treatments). Azoxystrobin was applied with a CO_2-propelled boom comprised of three flat fan nozzles (TeeJet 8004 XR VS Flat-Fan®, Spraying Systems Company, Wheaton, IL) calibrated to deliver 812 L ha^{-1}. Following azoxystrobin application, irrigation was withheld for three d, then supplemented to sustain turfgrass health. Plots were then mown at 0, 1, 2, 3, 7 or 14 d after treatment (DAT) and visually rated weekly for brown patch (% severity) through 28 DAT. A no mow treatment was added in 2019. Plots were mown every 7 DAT and clippings were mulched and returned.

2.3 Influence of Turfgrass Clipping Management Practices

Research was initiated June 14, 2018 and repeated on July 16, 2019 at Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC to investigate the influence of turfgrass mowing-clipping management practices following azoxystrobin application. Three d prior to initiation, tall fescue was mown (9.5 cm). Azoxystrobin application parameters were identical to aforementioned experiment. Following azoxystrobin application, irrigation was withheld for three d, then supplemented to sustain turfgrass health. At 3, 10 and 17 d after treatment (DAT), all turfgrass plots were mown, and their respective clippings were either mulched and returned to the canopy (i.e., returned), or removed from the area (i.e., collected), using a mower baggar lined with a plastic

bag (49 L; HDX Drawstring Kitchen Bags, Home Depot, Atlanta, GA). Plots were visually rated weekly for brown patch (% severity) through 28 DAT.

2.4 Sample Collection and Preparation

Turfgrass core samples (10.8 cm diam; 92 cm^2) were collected for residue analysis at 7, 14 and 21 DAT for mowing timing experiments and at 3, 7, 14, and 21 DAT for clipping management practice experiments. Turfgrass collected cores were segmented into two matrices; remaining aboveground vegetation and soil (0.0 to 2.5-cm depth). Sampling and core segmenting equipment were sterilized with ammonia:water (2:1 v/v) solution between each sample collection and segmentation to prevent contamination. Following segmentation, all samples were weighed and stored at -12° C until homogenization. Remaining aboveground vegetation tissue samples were milled and homogenized (Cuisinart Compact Portable Blending/Chopping System Model CPB-300; Conair Corp., Compact Blender; Stamford, CT) with dry ice and stored at -12° C until extraction and residue analysis. Soil samples were milled and homogenized [1.7 mm (Fitzmill Homoloid Model JT 6; Fitzpatrick Co., Elmhurst, IL)] with dry ice and stored at -12° C until extraction and residue analysis.

2.5 Residue Analysis

Azoxystrobin residue soil extraction and quantification for soil and vegetation were conducted via modifications to Jones & Earl (2002) and (Sundravadana et al. (2008), respectively. Azoxystrobin residue analysis was conducted via high performance liquid chromatography with a diode array detector (HPLC-DAD-MS) at the North Carolina State University Pesticide and Trace Elements Laboratory. A 10 g homogenized subsample mixed with 30 mL acetonitrile and shaken

[300 rpm(KS 501 Digital Shaker; IKA Works, Inc., Wilmington, NC)] for 30 min. A 10 mL aliquot was centrifuged (10 min), filtered (0.45 μm PTFE filter) and 1 mL was vialed for injection. Modifications to Sundravadana et al. (2008) for azoxystrobin vegetation extraction included: 10 g subsamples mixed with acetonitrile (30 mL) were added in glass jar (250 mL) and mixed using high speed homogenizer (Bio-Gen PRO200 homogenizer, Pro Scientific, Oxford, CT) for 5 min. Mixture was left to settle for 10 min then an aliquot (15 mL) was taken and centrifuged (3500 rpm) for 10 min. 1.5 mL were pipetted into Quechers tubes (Q-sep® QuEChERS dSPE tubes, Catalog No. 26123, Restek Corporation, Bellefonte, PA), vortexed (2 min), centrifuged (5 min), filtered (0.45 μm polytetrafluoroethylene filter) and 1 mL vialed for injection.

Azoxystrobin residue were quantified for both matrices by HPLC-DAD-MS (Agilent-1260 Infinity, Agilent Technologies, Incorporated, Wilmington, DE) equipped with a C_{18} silica column [75 mm length x 4.6 mm i.d. (Poroshell 120 EC-C_{18}; Agilent Technologies, Incorporated, Wilmington, DE]). HPLC parameters for azoxystrobin were 30°C column temperature; {[(acetonitrile:water) 7:3] + 0.1% formic acid by vol} mobile phase; 0.8 mL min-1 flow rate; 10 μL injection volume, and a 1.52 min retention time at 230 nm. Azoxystrobin limit of detection was 0.05 mg L^{-1} and limit of quantification was 0.25 mg L^{-1}, based on a 3:1 signal to noise ratio for both matrices. Pesticide residue was quantified using peak area measurements (Open LAB CDS ChemStation, Version C.01.04, Agilent Technologies, Incorporated, Wilmington, DE). Fortification checks ranged from 89 to 102%. Application verification pads were included for quality insurance and the recovery efficiencies for azoxystrobin were 109, 111 and 121% of the applied.

2.6 Experimental Design and Statistical Analysis

Both experiments included 3 replications and a non-treated control and were arranged in a randomized complete block design. For experiment 1, mowing timing after treatment (MTAT) and sample collection timing were considered fixed effects. For experiment 2, clipping management practice and sample collection timings were considered fixed effects. Means were separated using Fisher's LSD (p=0.05) using PROC GLM (Statistical Analysis Software, Version 9.4, SAS Institute, Cary, NC) to explore differences in azoxystrobin among turfgrass vegetation and soil over time. Data was pooled over years for mowing timing experiments (P = 0.6225) and clipping management practice experiments (P = 0.1411). Means were compared within a sample matrix (vegetation or soil) and within a sample collection timing.

3 Results

3.1 Mowing timing experiment

Sample collection timing (DAT) (P < 0.0001) and plant matrix (P < 0.0001) significantly influenced azoxystrobin residue in the tall fescue aboveground vegetation and soil. At 7 DAT, plots mown at 0 MTAT (immediately) resulted in the lower azoxystrobin residue in aboveground vegetation (14.1%) than when mown at 1, 2, 7, or not mown (22.7, 23.2, 21.2, 19.8% of applied, respectively) (Table 1.1). The 7 and 14 MTAT resulted in greater azoxystrobin (20.8 and 27.4% of applied), compared to 0, 1, and 2 MTAT (9.2, 10.8 and 12.3% of applied). By 21 DAT, azoxystrobin recoveries in the aboveground vegetation were similar with the exception of 0 MTAT resulting in less azoxystrobin than no mow plots (2.2 and 3.6% of the applied). In the soil of samples collected at 7 DAT, 0 DAT mow resulted in enhanced azoxystrobin (13.7%),

compared to mowing at 3 and 7 DAT, or no mow (8.4, 7.9, and 2.8% of applied), respectively (Table 1.2). In the soil at 14 DAT, azoxystrobin concentration were similar across treatments (9.1 to 10.9% of the applied). At 21 DAT, 7 MTAT increased azoxystrobin concentration (9.3%) in soil, compared to 0 and 1 MTAT (6.6 and 5.9% of the applied), respectively.

Weekly ratings for brown patch severity were conducted through 28 DAT, data from 2018 and 2019 were different and therefore data were not pooled. Additionally, both years were sorted by rating timing (DAT). In 2018, no differences were observed across mowing timing treatments, and disease severity ranged from 1.0 to 3.7% over the entire study (Table 1.3). Non-treated plots that did not receive azoxystrobin displayed increased levels of disease severity at 28 DAT (10%), compared to plots receiving azoxystrobin. In 2019, differences were not observed across mowing timing treatments, and disease severity ranged from 1.7 to 10.3% over the entire study (Table 1.4). Brown patch disease pressure was higher in 2019, where the non-treated plots expressed higher disease severity at 21 and 28 DAT (21.7 and 26.6%, respectively).

3.2 Clipping management practice experiment

Sample collection timing (DAT) ($P < .0001$) and sample matrix ($P < .0001$) influenced azoxystrobin residue in vegetation and soil. At 3 DAT, azoxystrobin concentrations were similar (36.8 and 38.2% of the applied) for collected and returned clipping treatments, respectively (Table 1.5). At 7 DAT, differences were observed between collected (13.8%) and returned (18.9% of applied) clipping treatments. By 14 DAT, residue in fescue vegetation was increased via returned clippings (17.7%) opposed to collected clippings (9.4% of the applied). Azoxystrobin residue in the vegetation was different at 21 DAT, returned clippings resulting 5.8% of the applied, compared to 4% when collected clippings.

Azoxystrobin concentrations were generally lower in the soil, compared to the aboveground vegetation and did not exceed 9.2% of the applied during the entire study (Table 1.6). Azoxystrobin in soil at 3 DAT was approximately twice as high in plots where clippings were returned (8%) compared to clippings removed (4% of the applied). By 7 DAT, similar azoxystrobin concentrations were observed when clippings were returned (8.1%) and collected (4.2% of applied). Azoxystrobin recovered at 14 were similar; however, <2% more of the applied was reported when clippings returned compared to collected. By 21 DAT, more azoxystrobin was in the soil when clippings were returned (8.0%) versus collected (4.8% of the applied).

Brown patch severity ratings were not significant across years; therefore, data were pooled over 2018 and 2019. Additionally, both years were sorted by rating timing (DAT). No visual brown patch severity differences were observed for collecting or returning treated clippings; however, at 21 and 28 DAT, the non-treated control had higher disease severity (10.8 and 16.7%) compared to the plots receiving azoxystrobin, ranging from 2.0 to 4.3% (Table 1.7).

4 Discussion

4.1 Mowing timing experiment

In the mowing timing experiment, depending on sample collection timing, delaying mowing after azoxystrobin application increased azoxystrobin residue found in the tall fescue aboveground vegetation and below ground soil. At 14 DAT, increased concentration of applied azoxystrobin were observed in the aboveground vegetation as MTAT is delayed, suggesting that delayed mowing may increase azoxystrobin retention in/on tall fescue vegetation. At 7 DAT,

differences are observed in MTAT in the soil matrix, where 0 DAT mow resulted in ~five-times more azoxystrobin (13.7%) recovery compared to no mowing treatment (2.8% of the applied). Data indicate that when turf is mown the same day as azoxystrobin application, more azoxystrobin residue is removed in clippings, and mulched and returned in the plot, resulting in increased soil residue. Similarly, when a plot is not mown, we observe less azoxystrobin residue in the soil, due to the lack of clipping removal and deposit onto soil. This is similar to Jeffries et al. (2016) who demonstrated that azoxystrobin can be removed in turfgrass clippings and can persist in clippings, where researchers removed (20% of applied) azoxystrobin in clippings from mowing tall fescue the same day as azoxystrobin application (Jeffries et al., 2016). By 21 DAT, minimal azoxystrobin was detected in the aboveground vegetation; however, 0 MTAT (2.2%) resulted in less azoxystrobin compared to no mow (3.6% of applied). The reported half-life for azoxystrobin is 72 to 164 d (USEPA, 1997), yet in this study, we observed shorter persistence ($T_{1/2}$ = 5.2 d) of azoxystrobin in tall fescue. Once applied in the field setting, azoxystrobin can dissipate, predominantly from photodegradation and secondarily dependent on microbial metabolism (USEPA, 1997). It is reported that azoxystrobin should photodegrade ($T_{1/2}$ = 11 days) in terrestrial environments, similar to the environment which our study was conducted (USEPA, 1997). Our research occurred during June and July where daylength was long, and microbial populations were active, potentially explaining the rapid dissipation of azoxystrobin we observed. However, our data are consistent with previous literature, where researchers observed 99% of the initial detected azoxystrobin, flutolanil, metconazole and pyraclostrobin were depleted in creeping bentgrass verdure by 14 d after application (Daniels & Latin, 2013).

Brown patch severity ratings were taken through 28 DAT; however, disease severity was considerably lower in 2018 than 2019. Therefore, we were not able to assess the influence of

these practices over 2 years. In 2018 and 2019, differences in brown patch severity were not observed across mowing timing treatments. This may be explained by the fact that azoxystrobin is a very efficacious fungicide against brown patch (Dernoeden and Krouse, 1998). Additionally, previous field report described azoxystrobin provided enhanced brown patch control (<10% severity) and for a long duration (35-d application intervals) (Settle and Fry, 2001). Koehler & Shew (2017) researched the effective concentrations (μg mL^{-1}) of azoxystrobin to inhibit 50% of radial mycelial growth *in vitro* of R. solani isolates. Researchers found that one isolate was inhibited by 50% with 5.13 (μg mL^{-1}). In a perennial turfgrass system, brown patch inoculum is produced when the pathogen is actively infecting the plant. This inoculum builds up over time from season to season, increasing incidence of infected plants. The increased azoxystrobin that is recycled when returning clippings may suppress disease development. Authors suggest that mowing timing should be delayed following an azoxystrobin application and that clippings should be returned.

4.2 Clipping management practice experiment

We hypothesized that returning clippings treated with azoxystrobin would increase azoxystrobin presence in tall fescue aboveground vegetation and belowground soil/associated roots, as well as decrease brown patch disease severity, compared to removing mown clippings. We observed an increase of azoxystrobin in/on the aboveground vegetation at 7, 14 and 21 DAT, when mown clippings were returned. Additionally, at 3, 7 and 21 DAT, more azoxystrobin was recovered in soil with returned clippings. Data suggest that the mown clippings contained azoxystrobin and when collected and removed from the turfgrass stand, a decreased concentration of the total initial azoxystrobin was observed. Plots were mown 3 times during the

experiment at 3, 10 and 17 DAT. These data agree with work conducted by Jeffries et al., 2016, where authors reported removing applied azoxystrobin from tall fescue (20%) when mown same day as application.

Settle and Fry (2001) researched the effect of clipping removal on brown patch development and reported brown patch incidence on tall fescue was not affected by collecting or returning clippings following azoxystrobin application; however, the experimental approach potentially limits confident elucidation on this management practice. More specifically, in their study, plots were treated with azoxystrobin (0.3 kg ai ha^{-1}) monthly from May to August during the evaluation period, which may have allowed the sequential applications to replenish azoxystrobin field residue that were depleted by routine clipping collection to an acceptable level for disease control (Settle and Fry, 2001). Without residue quantification over time in plots, it cannot be determined if mowing practices or sequential applications was the primary factor in acceptable brown patch control. In our study, we looked at the effect of removing or returning clippings on azoxystrobin efficacy, in addition to dissipation. As mentioned, increased concentrations of azoxystrobin were recovered in vegetation and soil when clippings were returned; however, similar to Settle and Fry (2001) we did not observe differences between clipping management treatments and their effect on brown patch severity. In 2018 and 2019, environmental conditions for strong disease pressure were not present. We hypothesize that under conditions favoring disease development, the additional azoxystrobin in the tall fescue system when returning mown clippings may increase potential for enhanced efficacy and less brown patch severity. Daniels & Latin (2013) looked at fungicide efficacy on *R. solani* inoculated turf samples in a bioassay experiment, where samples were incubated in a dew chamber. The bioassay results reported that creeping bentgrass is exposed to risk of brown patch

outbreak shortly (2 to 9 d) after fungicide application when environmental conditions favor pathogen development, a much shorter time frame than observed in the present study, under less favorable disease conditions. Future research should consider repeating these studies on a tall fescue host that has been inoculated with *Rhizoctonia* spp. prior to initiation, to determine if an increased brown patch severity may affect efficacy following mowing and clipping management practices. There is potential for extended azoxystrobin residue to provide disease suppression and the authors suggests returning clippings from an azoxystrobin treated area.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1.1 Influence of mowing timing on azoxystrobin recovery in tall fescue aboveground vegetation (0-9.5 cm) for 3 sample collection timings.

	Sample Collection Timings[a]		
MTAT[b]	7 DAT[c]	14 DAT	21 DAT
	---------- % of applied[d] ----------		
0	14.09 c[e]	9.24 c	2.15 b
1	22.67 a	10.82 c	3.23 ab
2	23.22 a	12.27 c	2.88 ab
3	18.23 b	13.07 bc	2.98 ab
7	21.23 ab	20.76 ab	3.05 ab
14	-	27.40 a	3.10 ab
No Mow	19.79 ab	13.85 bc	3.62 a

[a] Data pooled over two experimental runs.
[b] Irrigation was withheld after initial irrigation treatment through 3 DAT.
[c] Abbreviations: MTAT, mowing timing after treatment (days); DAT, days after treatment.
[d] Percent of nominal 0.6 kg ai ha^{-1} spray application rate.
[e] Means followed by same letter within DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 1.2 Influence of mowing timing azoxystrobin recovery in soil (0-2.5 cm) for 3 sample collection timings.

	Sample Collection Timings[a]		
MTAT[b]	7 DAT[c]	14 DAT	21 DAT
	---------- % of applied[d] ----------		
0	13.69 a[e]	10.12 a	6.55 bc
1	9.41 ab	10.45 a	5.92 c
2	10.41 ab	10.34 a	7.59 abc
3	8.40 b	10.87 a	8.94 ab
7	7.90 b	9.06 a	9.30 a
14	-	10.90 a	7.08 abc
No Mow	2.80 c	9.69 a	8.47 abc

[a] Data pooled over two experimental runs.
[b] Irrigation was withheld after initial irrigation treatment through 3 DAT.
[c] Abbreviations: MTAT, mowing timing after treatment (days); DAT, days after treatment.
[d] Percent of nominal 0.6 kg ai ha^{-1} spray application rate.
[e] Means followed by same letter within DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 1.3 Brown patch severity (%) as affected by MTAT and days after treatment in tall fescue, 2018.

	Days after treatment			
MTAT[a]	7	14	21	28
	---------- % brown patch[b] ----------			
0	1.7 a[c]	1.7 a	1.7 a	3.0 b
1	1.7 a	2.3 a	3.0 a	3.7 b
2	1.7 a	3.0 a	3.0 a	3.0 b
3	1.7 a	2.3 a	1.7 a	2.3 b
7	2.3 a	2.3 a	2.3 a	2.3 b
14	1.7 a	1.7 a	1.0 a	1.0 b
Control	2.3 a	6.0 a	6.0 a	10.0 a

[a] Abbreviations: MTAT, mowing timing after treatment (days).

[b] Rated as percent of plot area exhibiting symptoms (% severity).

[c] Means followed by same letter within DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 1.4 Brown patch severity (%) as affected by MTAT and days after treatment in tall fescue, 2019.

	Days after treatment			
MTAT[a]	7	14	21	28
	---------- % brown patch[b] ----------			
0	3.0 a[c]	6.0 a	4.0 b	5.6 b
1	4.3 a	4.3 a	3.7 b	6.0 b
2	7.0 a	10.33 a	8.7 b	6.6 b
3	2.3 a	4.3 a	3.7 b	5.3 b
7	2.3 a	6.0 a	5.0 b	6.0 b
14	1.7 a	3.7 a	5.3 b	7.0 b
No Mow	1.7 a	3.7 a	3.0 b	6.0 b
Control	3.7 a	11.7 a	21.7 a	26.6 a

[a] Abbreviations: MTAT, mowing timing after treatment (days).

[b] Rated as percent of plot area exhibiting symptoms (% severity)

[c] Means followed by same letter within DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 1.5 Effect of mowing clipping practices on azoxystrobin recovery in/on tall fescue aboveground vegetation (0-9.5 cm).

	Sample Collection Timings[a-c]			
Clipping practice	3 DAT	7 DAT	14 DAT	21 DAT
	---------- % of applied[d] ----------			
Returned	38.20 a[e]	18.89 a	17.73 a	5.76 a
Collected	36.78 a	13.77 b	9.37 b	4.01 b

[a] Data pooled over two experimental years.
[b] Mowing occurred at 3, 10 and 17 DAT and irrigation was withheld after initial irrigation treatment through 3 DAT.
[c] Abbreviations: DAT, days after treatment.
[d] Percent of nominal 0.6 kg ai ha^{-1} spray application rate.
[e] Means followed by same letter within DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 1.6 Effect of mowing clipping practices on azoxystrobin recovery in tall fescue soil (0-2.5 cm).

	Sample Collection Timings [a,b]			
Clipping practice	3 DAT[c]	7 DAT	14 DAT	21 DAT
	---------- % of applied[d] ----------			
Returned	8.09 a[e]	8.13 a	9.23 a	7.96 a
Collected	3.99 b	4.28 b	7.02 a	4.84 b

[a] Data pooled over two experimental runs.
[b] Mowing occurred at 3, 10 and 17 DAT and irrigation was withheld after initial irrigation treatment through 3 DAT.
[c] Abbreviations: DAT, days after treatment.
[d] Percent of nominal 0.6 kg ai ha^{-1} spray application rate.
[e] Means followed by same letter within DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 1.7 Effect of mowing clipping practices and days after treatment on brown patch severity (%).

Clipping practice [a]	Days after treatment			
	3	7	14	21
	% brown patch[b]			
Returned	2.8 a[c]	2.3 b	2.3 b	2.0 b
Collected	3.3 a	3.5 ab	4.3 b	2.8 b
Control	3.0 a	7.0 a	10.8 a	16.7 a

[a] Data pooled over two experimental runs.

[b] Rated as percent of plot area exhibiting symptoms (% severity).

[c] Means followed by same letter within DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$)

Chapter 2: Cyazofamid Distribution as Affected by Post-Application Irrigation Timing and Volume on a Bentgrass Putting Green

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Fungicides are necessary when managing root and crown diseases of turfgrasses. However, their physicochemical properties combined with the makeup of a USGA-specification putting green can impede movement into the root zone. In order to facilitate fungicide movement, turfgrass managers use various practices, including post fungicide application irrigation. Field research was initiated 2018 in Raleigh NC (Lake Wheeler Turfgrass Field Laboratory) and repeated in 2019 to quantify the effect of post-application irrigation timing and volume on cyazofamid soil distribution. Cyazofamid (1.1 kg ai ha^{-1}) was applied to unique plots on a USGA-specification putting green comprised of 'A1/A4' creeping bentgrass (*Agrostis stolonifera* L.). Following application, plots received either 0.3 or 0.6 cm irrigation immediately (i.e., 0 hours after treatment [HAT]), 6 HAT, or plots not irrigated at all (represented by 72 HAT). To quantify cyazofamid fate, plant and soil samples were collected 0 through 14 days after treatment (DAT). Turfgrass clippings were collected at 0, 1, 2, 3, 5, 7, 10 and 14 DAT via walk-behind reel mower to quantify residue loss via clipping collection. Additionally, at 0, 1, 5, 7 and 14 DAT, 10.8 cm diameter cores were collected and divided into four unique segments: verdure/thatch, as well as 0 to 2.5 cm, 2.5 to 5 cm and 5 to 7.5 cm soil depths. Across sample timings, immediate irrigation (i.e., 0 HAT) resulted in greater movement of cyazofamid through the verdure/thatch and into the soil-rootzone. Delaying irrigation or not irrigating at all (i.e., 6 HAT and 72 HAT) resulted in greater cyazofamid concentrations in the verdure/thatch layer, and less in the soil depths. Data generated as a result of this research will enhance turfgrass best management practices intended to

enhance fungicide vertical movement to the rootzone and provide information pertaining to cyazofamid dissipation kinetics from golf course putting greens.

Abbreviations: DAT, days after treatment; HAT, hours after treatment; PAIT, post-application irrigation timing

1 Introduction

Golf course superintendents are expected to provide superior aesthetics and playability on putting greens. Root and crown diseases commonly occur on putting greens, especially those located in regions that experience prolonged high humidity. *Pythium* spp. cause numerous diseases of turfgrasses; however, crown and root diseases caused by *Pythium* spp. are most often associated with highly maintained putting greens (Smiley et al., 2005). Several species have been recognized as members of the *Pythium* genus, found in association with creeping bentgrass (*Agrostis stolonifera* L.) roots and cause extensive damage, particularly during heat and physiological stress. *Pythium* spp. closely resemble fungi; however, they are taxonomically classified as stramenopiles. *Pythium* spp. produce oospores and their occurrence is prevalent in prolong humid or wet conditions. A common root disease that spawns from *Pythium* spp. includes *Pythium* root rot, characterized by root and crown deterioration, resulting in distinct patches or large irregular areas (Abad et al., 1994). Affected plant parts such as the crown, roots and stolons will appear dark and greasy. *Pythium* root rot is a persistent problem in poorly drained soils but can also occur in well-drained soils following extensive rainfall or irrigation and has been reported spreading along drainage patterns (Kerns & Butler, 2018). Adequate surface and subsurface drainage are imperative for managing *Pythium* root rot. Additionally, a combination of other best management cultural practices to limit turf stress and ultimately to mitigate *Pythium* root rot development include proper irrigation, aerification and topdressing, and increased air movement and sunlight penetration. Although useful, cultural practices alone will not reduce disease severity to maintain superior playability.

Fungicides are routinely applied to golf course putting greens and are able to reduce the activity and mitigate damage caused by pathogens. Pathogenic fungi are known to infect above and belowground plant parts. Foliar pathogens are easier to manage with fungicide applications because the majority of the active ingredient is directly applied within the zone of infection and disease. Root diseases however are very different as fungicides don't move down when applied to the foliage. Therefore, it is impossible to get 100% of the active ingredient to the target site where the pathogen resides. Fungicide applications on root diseases are often inconsistent and do not ensure adequate disease management (Latin, 2011). The turfgrass profile protects root pathogens, shielding them from contact from spray-applied fungicides. Without the active ingredient coming into contact with the pathogen, disease mitigation cannot be achieved. Gardner and Branham (2001) investigated the effect of turfgrass cover, compared to bare soil, on the effect of mefenoxam and propiconazole mobility and dissipation and concluded that existing turfgrass cover significantly decreased the amount of propiconazole that distributed into the soil layers. Fungicides that are registered to have activity on root and crown pathogens are commonly described as either local or acropetal penetrants (Latin, 2011). Local penetrant fungicides are able to move across plant tissue and acropetal fungicides are able to translocate up the plant through the xylem. Without the ability to translocate the active ingredient basipetally into contact with the pathogen, disease development is not effectively managed. In order to better ensure disease management, the applied fungicide must be distributed through the thatch and roots of a turfgrass plant, into closer contact with soilborne pathogens.

A common fungicide used on bentgrass putting greens, cyazofamid [4-chloro-2-cyano-N,N-dimethyl-5-p-tolylimidazole-1-sulfonamide], is used for control of *Pythium* diseases (USEPA, 2004). Most species of *Pythium* are sensitive to cyazofamid, making it arguably the best

Pythium root rot fungicide on the market (Hampy, 2020). Cyazofamid is a local penetrant fungicide, commonly used for preventative root rot suppression. Cyazofamid belongs to the QiI fungicide class and is able to interfere with electron transport during mitochondrial respiration, essential for pathogen energy and growth (Morton & Staub, 2008). Cyazofamid has a short field half-life (aerobic soil $T_{1/2}$ = 5.5 d), low water solubility (K_s = 0.1 mg L^{-1}), and depending on soil type, a moderate to high soil-organic carbon partition coefficient (K_{oc} = 657 to 1525 mL g^{-1}) suggesting limited movement, especially in a turfgrass setting containing high organic carbon (USEPA, 2004) (Table 2.1). Previous research confirms its short persistence and limited soil mobility by reporting cyazofamid did not move beyond 15 cm depth, despite delivering 60 cm water over a 5-day period in sandy loam and silty clay loam soils (Singh & Tandon, 2015).

Physicochemical properties of pesticides such as half-life ($T_{1/2}$), solubility (K_s) and sorption affinities (K_d and K_{oc}) offer an indication how a pesticide may persist and move in a soil matrix (Wauchope et al., 2002). Most fungicides can be classified as having a low to medium water solubility, and a moderate to high affinity to bind to organic matter, a combination of properties that limits their ability to move in soil (Ou & Latin, 2018). Hockemeyer and Latin (2015) showed that among the turfgrass system components, azoxystrobin, propiconazole, thiophanate-methyl, and pyraclostrobin remained mostly in the verdure/thatch, especially for compounds with high K_{oc} values. Cisar and Snyder (1996) found the majority of chlorpyrifos applied to a USGA bermudagrass putting green were retained in the thatch layer. The physicochemical properties possessed by fungicides readily allow for residue binding in the thatch and soil rootzone, which contain high (~10%) amounts of organic material. Singh and Tandon (2015) reported the presence of organic matter affects the movement of pesticide because of its high sorption affinity to soil. In order to enhance fungicide vertical distribution, researchers have evaluated a number of different

management practices. Some of these tactics include increasing carrier spray volume, soil surfactant use, pesticide injection, and post fungicide application irrigation, to name a few.

Wong and Corza (2005) observed summer patch control with azoxystrobin and pyraclostrobin as affected by application volume (815 and 1628 liter ha^{-1}) and determined no difference in disease control. Numerous studies have been conducted researching the effect of soil surfactants on fungicide downward movement. On a USGA creeping bentgrass putting green, Latin and Ou (2018) found there was no measurable effect when using a wetting agent on azoxystrobin, propiconazole, pyraclostrobin, and thiophanate-methyl distribution in the turf profile. More recently, in a ^{14}C laboratory experiment, Hutchens et al. (2020) found that soil lysimeters not treated with a soil surfactant retained at least 19.4% more myclobutanil in the top 2.5 cm of soil than when treated with a soil surfactant, effectively showing that soil surfactant inclusion increased myclobutanil vertical movement. Fidanza et al. (2005) experimented with high pressure injection of azoxystrobin and flutolanil, observing acceptable type-I fairy ring control within these plots compared to plots receiving flutolanil alone. Across previous lab and field experiments, evaluating soil lysimeters and putting greens, possibly the most effective tactic to aid fungicide movement is post application irrigation. A substantial amount of literature exists regarding the effect of irrigation on fungicide movement (Schumann et al., 2000; Gardner & Branham, 2001; Latin & Ou, 2018; Hockemeyer & Latin, 2015; Hutchens et al., 2019). Schumann et al. (2000) applied fenarimol, propiconazole and triadimefon to Kentucky bluegrass (*Poa pratensis* L.) followed immediately with 1.3 cm of irrigation. Despite post-application irrigation, authors reported no fungicide detected below the top 5.1 cm of soil. Gardner and Branham (2001) observed no influence of irrigation on propiconazole; however, mefenoxam was detected at deeper depths. Latin and Ou (2018) applied 0.51 cm of irrigation following fungicide applications and

found the majority of propiconazole, azoxystrobin, pyraclostrobin, thiophanate-methyl and carbendazim were recovered in the thatch; however, greater fungicide residue levels were found in the roots and soil when post application irrigation was applied. Hockemeyer and Latin (2015) observed that measurable amounts of fungicide were present in all turf sample components (verdure, thatch, roots, and sand) within 5 hours of application using 0.35 cm irrigation following fungicide application.

These data suggest that best management practices may promote fungicide distribution, potentially resulting in effective root disease management; however, they are not infallible. Acropetal and local penetrant fungicides, commonly registered for having activity on *Pythium* spp., are unable to translocate basipetally in the plant, emphasizing the need for their distribution throughout the thatch and rootzone. However, the high carbon content in putting greens combined with fungicide physicochemical properties results in high sorption of the fungicide to the available binding sites often abundant in organic soil particles, severely limiting their ability to vertically distribute. Cyazofamid label suggests 0.3 cm irrigation following cyazofamid application, however; the timing of irrigation following application is not suggested. Further research is needed to investigate irrigation practices that will increase fungicide movement throughout the rootzone, in order to suppress virulent soilborne pathogens, such as *Pythium* spp. The objective of this study was to examine the influence of irrigation timing and volume on cyazofamid vertical distribution in a creeping bentgrass putting green over time.

Core Ideas

- Fungicide distribution through the verdure/thatch layer and into the rootzone is limited.
- Immediate irrigation following application enhanced cyazofamid vertical movement.

- Closer contact between fungicide and pathogen may enhance disease suppression.

2 Material and Methods

Field experiments were initiated on June 5, 2018 and May 30, 2019 at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, conducted on an 'A1/A4' creeping bentgrass putting green constructed with rootzone and soil physical properties consistent with USGA recommendation (USGA, 2004). The rootzone had a pH of 6.1 and approximately 3.3% organic matter w/w. Prior to initiation, the experimental area was mowed 6 d per week at a 3.8 mm height of cut. Fertilizer was applied to deliver N at 130 kg ha^{-1} yr^{-1} and K at 60 kg ha^{-1} yr^{-1}. Up until 1 d prior to initiation, irrigation was applied as needed to prevent wilt. Cyazofamid was not applied to research areas for 2 yr prior to initiation. Additionally, soil and vegetation samples from the research area were analyzed prior to initiation to confirm cyazofamid residues were non-detectable.

One day prior to trial initiation, areas were mown with a walk-behind reel mower, (Toro Greensmaster Flex 21®, The Toro Company, Bloomington, MN) clippings were collected, and soil was irrigated to field capacity. At initiation (9:00 AM EST), cyazofamid (Segway®; PBI/Gordon Corp., Kansas City, MO) was applied at the current single application maximum rate (1.1 kg ai ha^{-1}) to unique plots measured at 0.9 by 3.6 m with alleys between replications (0.9 m) and treatments (0.3 m) to assist with clipping sample collection. Cyazofamid was applied with a CO_2-propelled equipment comprised of a single flat-flan nozzle (TeeJet AI9508E VS Flat-Fan®, Spraying Systems Company, Wheaton, IL) calibrated to deliver a carrier volume of 812 L ha^{-1}. Following cyazofamid application, plots received either 0.0, 0.3, or 0.6 cm irrigation (by calibrated H_2O flow meter) either immediately (0 HAT), after a 6-hour drying period (6 HAT), or not irrigated at all

(represented by 72 HAT). Normal irrigation resumed on all plots at 3 DAT to sustain turfgrass health and for the remainder of the experiment, rootzone moisture levels were monitored with and kept between 12 – 20% (FieldScout TDR 300, Spectrum Technologies Inc., Aurora, IL).

2.1 Sample Collection

To quantify cyazofamid residue in the turfgrass system, plant and soil samples were collected through 10 DAT. At 0, 1, 2, 3, 5, 7 and 10 days after treatment (DAT), turfgrass clippings were collected from unique plots via walk-behind reel mower to quantify residue loss via clipping collection. At 0 DAT, clippings were collected 2 hrs after 0 HAT and 6 HAT irrigation events. Clippings were collected in the reel mower clipping catch-basket lined with a plastic bag (49 L; HDX Drawstring Kitchen Bags, Home Depot, Atlanta, GA), fresh mass was recorded (g) , and samples were homogenized (Cuisinart Compact Portable Blending/Chopping System Model CPB-300; Conair Corp., Compact Blender; Stamford, CT) with dry ice and stored at -12°C until extraction and residue analysis. Additionally, at 0, 1, 5, 7, and 14 DAT, core sample collections (10.8 cm diam; 91.6 cm^2) were collected from unique plots. Between each sample collection, sampling equipment was sterilized with ammonia:water (2:1 v/v) solution and dried with napkins to prevent contamination. Core samples were horizontally dissected into 4 segments: remaining aboveground vegetation, as well as 0 to 2.5 cm, 2.5 to 5 cm and 5 to 7.5 cm soil depths. Segmenting equipment was sterilized with ammonia:water (2:1 v/v) solution and dried with napkins to prevent residue transfer. Following collection and segmentation, all samples were frozen, weighed, homogenized (Fitzmill Homoloid Model JT 6; Fitzpatrick Co., Elmhurst, IL) with dry ice and stored at -12°C until subsampling, extraction and residue analysis to quantify pesticide residue. To determine organic matter content of the putting green sample, subsamples of the turfgrass sample

components were taken and oven dried at 105°C before igniting in a muffle furnace for 2 hours at 360°C. The percent weight loss during the ignition step was reported as % organic matter w/w.

2.2 Residue Analysis

Cyazofamid residue extraction and quantification for soil and turfgrass clippings were conducted via modifications to methods by Tandon and Singh (2012) and Lee et al. (2014), respectively. Cyazofamid residue analysis was conducted via high performance liquid chromatography with a diode array detector (HPLC-DAD-MS) at the North Carolina State University Pesticide and Trace Elements Laboratory. A 10 g homogenized soil was mixed with 30 mL acetonitrile in a 250-mL conical tube and contents were shaken shaken [300 rpm(KS 501 Digital Shaker; IKA Works, Inc., Wilmington, NC)] for 30 min. A 10 mL aliquot was taken and centrifuged (3500 rpm) for 10 min, and 1 mL filtered (0.45 µm PTFE filter; Thermo Fisher Scientific, Inc., Pittsburgh, PA) syringed into HPLC vial. For vegetation, 10 g homogenized vegetation was weighed into in a 500-mL glass jar then 300 mL acetonitrile was added and mixed using high speed homogenizer (Bio-Gen PRO200 homogenizer, Pro Scientific, Oxford, CT) for 5 min. The mixture was left to settle for 10 min then an aliquot of 10 mL was taken and centrifuged (3500 rpm) for 10 min. 1.5 mL was taken from centrifuged samples and was loaded onto Quechers tubes (Q-sep® QuEChERS dSPE tubes, Catalog No. 26123, Restek Corporation, Bellefonte, PA), vortexed (2 min), centrifuged (5 min), then 1 mL was syringe filtered (0.45 µm PTFE filter) and vialed for injection.

Cyazofamid residues were quantified for both matrices by HPLC-DAD-MS (Agilent-1260 Infinity, Agilent Technologies, Incorporated, Wilmington, DE) equipped with a C_{18} silica column [75 mm length x 4.6 mm i.d. (Poroshell 120 EC-C_{18}; Agilent Technologies, Incorporated,

Wilmington, DE]). High performance liquid chromatography method parameters for vegetation and soil include: 30°C column temperature; (acetonitrile:water (60:40 v/v) + 0.1% formic acid by vol) mobile phase; 0.8 mL min^{-1} flow rate; 10 µL injection vol, and retention time was 3.88 min at 279 nm wavelength. Limit of detection and quantification were 0.025 and 0.05 mg L^{-1} respectively. Pesticide concentrations were quantified using peak area measurements (OpenLAB CDS ChemStation, Version C.01.04; Agilent Technologies, Inc., Wilmington, DE, USA). Residue concentrations above the calibration curve were diluted and re-injected for analysis. Finally, fortification recovery checks for vegetation and soil matrices ranged from 86 to 102%. Application verification pads were included for quality insurance and the recovery efficiencies for cyazofamid were 116, 118 and 121% of the applied.

2.3 Experimental Design and Statistical Analysis

Experiments included 2 irrigation amounts (0.3 or 0.6 cm) and 2 irrigation timings (0 or 6 or HAT). In 2019 run, a no-irrigation treatment was added, referred to as 72 HAT, where plots only received irrigation after 3 DAT, for plant health purposes. Experiments included 3 replications and were arranged in a randomized complete block design, blocked by replication. Samples were taken from the same unique plot during each sample collection timing. Data were subject to ANOVA ($P = 0.05$) and means were separated using Fisher's Protected LSD ($P<0.05$) using PROC GLM (Statistical Analysis Software, Version 9.4, SAS Institute, Cary, NC) to evaluate differences in fungicide distribution over time and among turfgrass components.

3 Results

On the research area putting green, organic carbon content was 6.6, 5.2, 4.4, and 2.0% w/w for verdure/thatch, 0 to 2.5 cm, 2.5 to 5 cm, and 5 to 7.5 cm, respectively (Table 2.2).

Difference in cyazofamid concentration were observed across all turfgrass components (clippings, verdure/thatch, 0-2.5, 2.5-5, 5-7.5 cm soil depths) for sampling timing (DAT) and irrigation timing. Means were separated within turfgrass components ($P < 0.0001$), within sample timing ($P < 0.0001$), and will be presented accordingly. Irrigation timings were significant; therefore, irrigation volumes were pooled. Irrigation at 72 HAT resulted in more cyazofamid residue in the turfgrass clippings (1.7% of applied) compared to 0 HAT or 6 HAT irrigation at 0 DAT (Table 2.3). Similar results were observed at 1 DAT, where irrigation 72 HAT resulted in greater removal of analyte (0.7%) compared to irrigation 0 HAT and 6 HAT (0.4% of the applied). At 2 and 3 DAT, $\leq 0.4\%$ of the applied cyazofamid was found in any irrigation timing, and differences were not observed. By 5 and 7 DAT, more cyazofamid was recovered when irrigated 6 or 72 HAT, but detection was minimal.

At 0 DAT, more cyazofamid was recovered in the verdure/thatch when irrigated 6 HAT or 72 HAT (94.8% and 98.1% of the applied, respectively), compared to irrigating 0 HAT (68.7% of the applied)(Table 2.4). Similar results were observed at 1 DAT in the verdure/thatch with plots receiving irrigation 0 HAT resulting in $\geq 24.3\%$ less of the applied cyazofamid residue, compared to irrigation 6 or 72 HAT. By 5 DAT, 22.0, 36.4, and 43.7% of the applied was detected in plots irrigated 0 HAT, 6 HAT and 72 HAT, respectively, meaning that more cyazofamid was moved below the verdure/thatch layer when irrigation occurred closer to cyazofamid application. At 7 DAT, 0 HAT irrigation resulted in less cyazofamid (15.7%), compared to 6 HAT (21.2%) and 72 HAT irrigation (24.3% of the applied). Similar results were observed at 14 DAT, where irrigation 0 HAT resulted in less than half (3.8%) the amount of cyazofamid that is found irrigating at 6 and 72 HAT (9.0 and 8.1% of applied). A steady decline

of cyazofamid concentration in the verdure/thatch is observed over sampling days, as it degrades by photolysis and soil microorganisms in aerobic conditions (USEPA, 2004).

In the first depth of the soil (0 to 2.5 cm), greater than 5-times more cyazofamid was recovered when irrigated 0 HAT (29.3%), compared to 6 HAT (5.13% of the applied)(Table 2.5). Similar results were observed at 1 DAT, where there is a four-fold increase of cyazofamid when plots were irrigated 0 HAT (27.3%) compared to 6 HAT (6.4% of the applied). In this same depth, there was no detection of cyazofamid at 0 or 1 DAT when irrigation occurred 72 HAT. By 5 DAT, 11% more of the applied cyazofamid was recovered when irrigated 0 HAT compared to 6 HAT. By 7 DAT, irrigation 0 HAT still resulted in greater cyazofamid (<2% more of the applied) compared to 6 or 72 HAT. At 14 DAT, the cyazofamid moved below 0 to 2.5 cm when irrigated 0 HAT, resulting in greater cyazofamid recoveries associated with irrigation 6 and 72 HAT. In the 2.5 to 5 cm soil layer, only irrigation 0 HAT resulted in cyazofamid detection at 1 DAT, however it was below the limit of quantification. By 5 DAT, 0 HAT irrigation resulted in 0.8% of applied and was detected in plots irrigated 6 HAT, however detection was below limit of quantification. 72 HAT irrigation did not result in any cyazofamid detection in the 2.5-5 cm soil layer until 14 DAT, but was <LOQ, less than both 6 HAT (1%) and 0 HAT (1.9% of the applied) irrigation. In the 5 to 7.5 cm soil layer, detection of cyazofamid was observed at 5, 7 and 14 DAT when irrigated 0 HAT or 6 HAT; however, the concentration was below the limit of detection. No cyazofamid was detected in this layer through the entire experiment if not irrigated within 72 HAT.

4 Discussion

Overall, experiment results generally agree with previous reports that fungicide removal in clippings is minimal and movement below the verdure/thatch layer is limited, even with post application irrigation (Cisar & Snyder, 1996; Latin & Ou, 2018; Hockemeyer & Latin, 2015; Lickfeldt & Branham, 1995; Larsbo et al., 2008). Cisar and Snyder (1996) evaluated the quantity of chlorpyrifos in turf clippings, thatch, soil and percolate water. Of the applied chlorpyrifos liquid formulation, researchers observed less than 1% were removed in the clippings and the majority on residue was retained in the thatch layer, which makes sense considering its high K_{oc} (6,070 mL g^{-1}) and low water solubility (2 mg L^{-1}) (National Center for Biotechnology Information, 2021). These results agree with our data regarding cyazofamid removed in clippings, where a maximum of 1.7% of applied cyazofamid was removed in clippings for any treatment at 0 DAT. Rapid loss of cyazofamid in the clippings was clearly observed over time, and by 10 DAT, cyazofamid detection was below the LOQ. Pitts et al. (1995) evaluated the degradation of chlorothalonil, metalaxyl, triadimefon, and vinclozolin in bentgrass clippings, where researchers observed high fungicide initial concentrations coupled with rapid loss, reporting half-lives of 3.2, 4.4, 1.1 and 2.6 days, respectively. Similar to Pitts et al. (1995), cyazofamid persistence in the clippings was fleeting, where we observed no detection by 14 DAT. Lickfeldt & Branham (1995) reported that in a Kentucky bluegrass stand, both leaves and thatch were strong sorbents for organic compounds and may have significant impact on the sorption and overall fate of pesticides applied to turf.

Regardless of irrigation timing, the majority of cyazofamid was retained in the verdure/thatch layer. Beard (1973) described the 'thatch' as a layer of organic matter that is "a tightly intermingled layer of dead and living stems and roots that develops between the green vegetation and the soil surface". Ledeboer and Skogley (1967) reported that the thatch has a high

carbon content, large macropores and low bulk density relative to soils, a recipe for facilitating significant pesticide sorption (Dell et al., 1994). Ou and Latin (2018) evaluated influence of management practices on distribution of azoxystrobin, fluxapyroxad, propiconazole, and pyraclostrobin applied to a creeping bentgrass putting green. Authors reported that the majority of fungicide was captured by verdure/thatch, and that fungicide concentration was 1 to 2 orders of magnitude higher in the verdure/thatch compared to other turf components. In particular, researchers observed the greater accumulation of pyraclostrobin in the verdure/thatch layer, likely due its high K_{OC} value (9315 mL g^{-1}) and low water solubility (1.9 mg L^{-1}) since organic compounds with high sorption coefficients tend to more tightly bind to organic matter, and less likely to solubilize. The K_{OC} values for cyazofamid range from 657 to 1525 mL g^{-1}, classified as moderate to high, and its water solubility is 0.1 mg L^{-1}, considered very low (Table 2.1), suggesting limited vertical distribution. Cyazofamid physicochemical properties combined with the high organic carbon content in the verdure/thatch layer (6.6% w/w, Table 2.2) readily facilitated sorption of cyazofamid, further explaining its limited movement.

Despite cyazofamid concentrating in the verdure/thatch layer, greater movement through the thatch into the soil layers was observed when irrigation was applied immediately following application. Irrigation closer to the time of fungicide application, gave less time for the fungicide to dry and sorb to turf and thatch components. Irrigation kept the fungicide as part of an aqueous solution, acting as a physical transport for the molecule, allowing gravity to move the fungicide down into the profile. Latin and Ou (2018) also observed generally higher fungicide residue levels were associated with post-application irrigation in the roots and soil, where they applied 0.51 cm irrigation immediately after fungicide application. Similarly, Hockemeyer and Latin (2015) observed azoxystrobin, propiconazole, thiophanate-methyl and pyraclostrobin in all turf sample

components (verdure, thatch, roots, and sand) within 5 hours of application using 0.35 cm irrigation following fungicide application. Gardner and Branham (2001) researched the effect of high (~5 cm wk^{-1}) and low (~3 cm wk^{-1}) irrigation regimes on propiconazole and mefenoxam and observed no influence of irrigation on propiconazole; however, mefenoxam was detected at deeper depths. Results from this study corresponds with listed physicochemical properties associated with propiconazole and mefenoxam, where propiconazole has a very high soil organic carbon sorption coefficient (K_{oc} = 960 mL g^{-1}) and moderate water solubility (K_s = 150 mg L^{-1}), while mefenoxam has a moderate K_{oc} (20-790 mL g^{-1}) and a very high-water solubility (K_s = 26,000 mg L^{-1}). More specific to cyazofamid, previous research was conducted to evaluate dissipation kinetics and leaching of cyazofamid in texturally different agricultural soils (Singh & Tandon, 2015). Researchers applied 120 mL irrigation every 24 h for 5 d and observed that cyazofamid remained in the top 15 cm depth, regardless of bare sandy loam or silty clay loam soil types. Although limited, cyazofamid vertical movement in the study by Singh and Tandon (2015) was greater than movement observed in our experiments. This may be attributed to the existing verdure and thatch layer, which was non-existent in the Singh and Tandon study. Movement of cyazofamid was increased in the rootzone when irrigated 0 HAT; however, by 14 DAT, less than 2%, and 0.4% of the applied was recovered in the 2.5 to 5 cm and 5 to 7.5 cm soil depths, respectively. Considering the organic carbon content of the soil depths (Table 2.2), it is reasonable that only minor amounts of the applied cyazofamid are able to make it to these depths. These data are similar to results seen by Larsbo et al. (2008), who conducted an experiment evaluating the effect of root zone composition on fungicide leaching from golf greens. Researchers observed that leaching of iprodione, azoxystrobin and propiconazole was near zero for lysimeters constructed with a greens-mix soil medium, compared to greater leaching observed in a straight sand lysimeter. They

concluded the high organic matter content enabled fungicide sorption to these particles, as well as degradation of the fungicides may be been faster in greens-mix soil due to higher microbial activity.

Although present experiments and previous literature has shown that vertical distribution of fungicides is difficult to achieve in systems containing high organic carbon, our research suggests that applying immediate irrigation will help facilitate fungicide movement. We recommend turfgrass managers irrigate immediately or as soon as possible following a fungicide application in order to maximize distribution. More fungicide movement into the rootzone, even if limited, will increase the contact of the fungicide with existing pathogens, potentially increasing fungicide efficacy and overall pathogen suppression. In a recent study, researchers evaluated in vitro sensitivity of mycelium of Pythium spp. isolates and determined EC_{50} concentrations for commercially available fungicides (Hampy, 2020). They discovered that as little as 0.002 ppm of cyazofamid was able to suppress certain *Pythium* isolates, indicating that small amounts of active ingredient may have a significant effect on *Pythium* suppression. Our goal for turfgrass managers is to increase the fungicide efficacy and consistency when applied, and this research suggests that post application irrigation may help facilitate. Conducted research provides insight on enhancing cyazofamid vertical distribution and can potentially be extrapolated to other fungicides considering it physicochemical properties that suggest limited mobility. Additional future research to examine effects of management practices on fungicide fate will not only increase our understanding of fungicide distribution, but also provide disease control on golf course putting greens.

Conflict of Interest

The authors declare no conflict of interest.

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Table 2.1 Cyazofamid classifications and chemical properties.

Fungicide[a]	**Fungicide class**	**Phytomobility**	**K_{oc}**[b]	**K_s**[c]	**$T_{1/2}$**[d]	**Application rate**
			mL g^{-1}	mg L^{-1}	days	kg ai ha^{-1}
Cyazofamid	Quinone inside inhibitor (QiI)	Local penetrant	657 to 1525	0.1	5.5 d	1.1

[a] USEPA, 2004.
[b]Soil organic carbon-water partitioning coefficient.
[c]Water solubility
[d]Aerobic soil half-life

Table 2.2 Creeping bentgrass putting green sample component organic carbon content values.

Sample matrix[a]	Organic carbon content %[b]
	wt/wt
Verdure/thatch	6.6
0-2.5 cm	5.2
2.5-5 cm	4.4
5-7.5 cm	2.0

[a] Samples collected June 2019 from research area and are representative from both years.
[b] Organic carbon weight determined via loss-on-ignition 360° C.

Table 2.3 Effect of post application irrigation timing on cyazofamid in creeping bentgrass clippings over time.

	Days after treatment							
Post-application irrigation timing[a]	0	1	2	3	5	7	10	14
	----------% of applied[b] ----------							
0 HAT [c]	0.54 b[d]	0.36 b	0.34 a	0.36 a	0.04 b	0.006 b	<LOQ	ND
6 HAT	0.74 b	0.44 b	0.44 a	0.39 a	0.08 a	0.02 a	<LOQ	ND
72 HAT	1.74 a	0.67 a	0.16 a	0.15 a	0.07 a	0.02 a	<LOQ	ND

[a] Data pooled over two experimental years and over three irrigation volumes (0.0, 0.3 or 0.6 cm H_2O).

[b] Percent of nominal 1.1 kg ai ha^{-1} application rate.

[c] Abbreviations: HAT, hours after treatment; LOQ, limit of quantification; ND, non-detect.

[d] Means followed by same letter within each DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 2.4 Effect of post application irrigation timing on cyazofamid distribution in verdure/thatch collected on a USGA bentgrass putting green.

	Days after treatment				
Post-application irrigation timing[a]	0	1	5	7	14
	----------% of applied[b] ----------				
0 HAT[c]	68.70 b[d]	62.77 b	22.00 c	15.67 b	3.76 b
6 HAT	94.77 a	87.13 a	36.36 b	21.16 a	9.03 a
72 HAT	98.10 a	92.13 a	43.68 a	24.34 a	8.14 a

[a] Data pooled over two experimental runs and over three irrigation volumes (0.0, 0.3 or 0.6 cm H_2O).
[b] Percent of nominal 1.1 kg ai ha^{-1} application rate.
[c] Abbreviations: HAT, hours after treatment. ¨
[d] Means followed by same letter within each DAT are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Table 2.5 Effect of post application irrigation timing on cyazofamid soil distribution on a USGA bentgrass putting green.

	Days after treatment [a]														
	---------- 0 ----------			---------- 1 ----------			---------- 5 ----------			---------- 7 ----------			---------- 14 ----------		
Depth (cm)	0 HAT [b]	6 HAT	72 HAT	0 HAT	6 HAT	72 HAT	0 HAT	6 HAT	72 HAT	0 HAT	6 HAT	72 HAT	0 HAT	6 HAT	72 HAT
	% of applied[c]														
0 to 2.5	29.3 a[d]	5.13 b	ND	27.32 a	6.42 b	ND	16.05 a	4.95 b	<LOQ	6.06 a	3.73 b	2.62 b	2.81 a	1.67 b	3.30 a
2.5 to 5.0	ND	ND	ND	<LOQ	ND	ND	0.81 a	<LOQ	ND	1.33 a	0.66 a	ND	1.86 a	0.96 b	<LOQ
5.0 to 7.5	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	<LOQ	<LOQ	ND	<LOQ	<LOQ	ND

[a] Data pooled over two experimental runs and over three irrigation volumes (0.0, 0.3 or 0.6 cm H_2O).
[b] Abbreviations: HAT, hours after treatment; LOQ, limit of quantification; ND, non-detect.
[c] Percent of nominal 1.1 kg ai ha^{-1} spray application rate.
[d] Means followed by same letter within each DAT and within Depth are not significantly different according to Fishers Protected LSD ($P < 0.05$).

Chapter 3: Effect of Soil Surfactant on Mefenoxam Distribution in Soil

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Research to date has shown that use of soil surfactants can enhance vertical pesticide movement. Mefenoxam is a commonly used fungicide for preventative disease suppression of *Pythium* spp. and its physiochemical properties (K_s = 26,000 mg/L; K_{OC} = 20 to 790) suggest it may readily distribute through the soil profile. In a laboratory experiment, ^{14}C-mefenoxam was quantified within soil column depths (0.0 to 2.5, 2.5 to 5.0, 5.0 to 7.5, 7.5 to 10, 10.0 to 12.5 and 12.5 to 15.0-cm; 90:10 sand:peat v/v) in an attempt to elucidate vertical distribution of mefenoxam when applied with the soil surfactant Cascade Plus. Cascade Plus was applied at 5.15 µL column^{-1} (2.34% vol/vol) to unique soil columns 24 hours prior to ^{14}C-mefenoxam (0.76 kg ai ha^{-1}; 186.4 µL column^{-1}) application. Immediately following ^{14}C-mefenoxam application, 0.635-cm irrigation was applied to all columns and soil collection occurred at 3, 7, 14 and 21 days after treatment (DAT). In the first run of the experiment, regardless of soil surfactant, the majority of ^{14}C-mefenoxam was detected in the 0 to 2.5-cm depth (85.0 to 100.0% of applied). Pooled over collection timings, more C^{14} mefenoxam was detected when applied with a surfactant (5.8% of applied), compared to 2.9% with no surfactant in the 2.5 to 5.0-cm depth. In the second run of the experiment, increased mefenoxam movement was observed from both surfactant treated soil and non-surfactant treated soil. Across sample timings, lysimeters not treated with a soil surfactant retained > 32% more mefenoxam in the top 2.5 cm of soil compared to soil surfactant treatment. Furthermore, more than 11% of mefenoxam was recovered in both the 5-7.5 and 7.5-10 cm sampling depth. Results from this experiment illustrate how a soil surfactant inclusion can fungicide distribution in soil when targeting root and crown diseases.

Introduction

The typical United States Golf Association (USGA) putting green is constructed with at least 10% w/w organic material mixed with sand. Turf in these systems is extremely dense and usually form an organic layer near the surface call thatch. Most fungicides, especially those used for preventative suppression of root and crown diseases in turf, have high affinity to sorb to soil particles (K_d) and organic carbon (K_{oc}). These high values readily allow for binding to organic matter and soil particles, which may render fungicides not bioavailable and ineffective against root pathogens. Laboratory studies show that turfgrass leaves and thatch strongly sorb organic compounds and thus may have a significant effect on pesticide fate applied to turfgrass (Lickfeld & Branham, 1995). When managing root and crown pathogens, it is imperative for the active ingredient to reach the pathogen in the root zone in order to suppress disease. Most pathogen activity is hypothesized to occur in the upper most portions of the rootzone nearest to the highest concentration of organic matter. Fungicide residues must come into contact with the pathogen for more efficacious disease inhibition, yet movement may be hindered by the dense organic material at or near the surface. Additionally, most fungicides are classified as acropetal penetrants, meaning that once absorbed in the plant, they are able to translocate upward through the xylem (Latin, 2011). Soilborne pathogens are located in the rootzone of the profile; however, without the ability of the plant to translocate the active ingredient into the roots, pathogen suppression is not achieved. There is a combination of challenges that precludes fungicide movement in a turfgrass profile; however, previous research has experimented with irrigation practices to enhance fungicide movement (Latin & Ou, 2018: Gardner & Branham, 2001; Hutchens et al., 2019). In a field trial, Latin & Ou (2018) observed post-application irrigation resulted little fungicide detected below the verdure/thatch layer of a USGA specification putting green. A field experiment by Gardner &

Branham (2001) studied the effect of irrigation on propiconazole and mefenoxam mobility and observed limited downward movement with the majority of the residue detected in the top 2 cm, regardless of irrigation treatment. Under controlled conditions, Hutchens et al. (2019) observed 51% and 56% of applied myclobutanil and tebuconazole remained in the top 5 cm of soil in lysimeters regardless of irrigation treatment. Literature suggests that fungicide vertical movement is limited in systems containing thatch or high amounts of organic material. Additionally, most fungicides cannot translocate downward; thus, other measures must be taken to aid fungicide movement and efficacy of root and crown diseases.

Pythium root rot (*Pythium* spp.) is a common root and crown disease observed on creeping bentgrass (*Agrostis stolonifera* L.) and ultradwarf bermudagrass (*Cynodon dactylon* L. Pers. x *C. transvaalensis* (BurttDavy)) putting greens primarily during periods of turfgrass physiological stress. *Pythium* root rot develops in extended periods of soil saturation, caused by poor drainage, excessive shade, over-irrigation, heavy rainfall and excessive thatch layers. Once temperatures rise, the heat and physiological stress can result in extensive damage of creeping bentgrass. Symptoms may appear as orange and yellow irregular patterns and may spread along drainage routes during periods of heavy rainfall; however, diagnosing root rot can be very difficult based solely on symptomology (Smiley et al., 2005). Research has demonstrated several *Pythium* species exist and have the ability to cause root rot at varied temperature ranges, making it difficult to correctly timing fungicide applications. This likely explains variability of fungicide efficacy that is reported among putting greens (Kerns & Butler, 2018). Furthermore, variability of fungicide efficacy results from binding to thatch and organic matter, prohibiting active ingredient distribution into the root zone where *Pythium* root rot pathogens are most active.

Mefenoxam is a commonly used preventative fungicide, specifically toxic against oomycete pathogens (*Pythium* spp.) and their cellular functions. Mefenoxam is an acropetal penetrant fungicide and once absorbed and diffused through the plant cuticle, it moves apoplastically around cells until translocated upward through the xylem (Latin, 2011). This lack of mobility is important because it further emphasizes the need for physical movement. Mefenoxam was chosen in this experiment part in due to its physicochemical properties. Mefenoxam has a very high-water solubility (K_s = 26,000 mg L^{-1}) and low to moderate soil-organic carbon partition coefficient (K_{oc} = 20 to 790 mL g^{-1}) (Liu et al, 2009; Lewis et al, 2016). These properties suggest moderate to high potential for soil mobility and overall vertical distribution. Previous research has evaluated environmental fate and movement of metalaxyl, a similar fungicide (Horst et al., 1996; Starrett et al., 1996). Horst et al. (1996) found >28% of applied metalaxyl distributed through the verdure/thatch and was recovered in soil when applied to Kentucky bluegrass (*Poa pratensis* L.) growing in a 60-cm soil column. Authors speculated that enhanced mobility of metalaxyl compared to pendimethalin and chlorpyrifos could be attributed to higher polarity and slow degradation of mefenoxam in soil. Starrett et al. (1996) found increased irrigation volume and frequency enhanced metalaxyl movement through a 50-cm soil column and into the leachate. Previous research illustrated rapid vertical movement of mefenoxam through a soil profile, regardless of existing turfgrass cover or irrigation regime (Gardner and Branham, 2001). Researchers observed movement of mefenoxam to the 15- to 30-cm soil section; however, the majority of mefenoxam was found in thatch of plots containing turfgrass or in the top 1 cm of soil on bare soil plots.

Soil surfactants are commonly applied to golf course putting greens to increase or decrease moisture retention in the soil profile. They are chemicals that cause a physical change of the surface

of liquids and depending on their specific chemical properties, surfactants have many other uses, such as emulsifiers, dispersants, spreaders, penetrants, stickers and detergents (Kostka et al., 1997). Soil surfactants reduce soil hydrophobicity and increase water infiltration into the soil profile (Karnok et al., 2004; Mitra et al., 2006). Previous research demonstrates that soil surfactants can increase turf quality and mitigate localized dry spot (LDS) (Cisar et al., 2000; Fidanza and Bagwell, 2005; Karnok et al., 2004). Previous research also shows that soil surfactants combined with selects fungicide enhanced fairy ring and brown patch control compared to fungicide alone (Fidanza et al., 2007; Kerns et al., 2018). Turf managers frequently apply soil surfactants prior to fungicide application, with the thought it may facilitate more downward movement of the fungicide. Results from previous research regarding use of soil surfactant to promote fungicide movement has varied. In a field experiment, Latin (2018) found no measurable effect on fungicide distribution in the turf profile when a wetting agent was applied 24 hrs prior to fungicide application. Other research concluded soil surfactants enhanced pesticide downward distribution (Hutchens et al., 2020; Gannon et al., 2017). In a ^{14}C laboratory experiment, Hutchens et al. (2020) found that soil lysimeters not treated with a soil surfactant retained at least 19.4% more myclobutanil in the top 2.5 cm of soil compared to soil surfactant treatment. Furthermore, 14% more of the recovered myclobutanil was detected in the 5-7.6 cm depth when a soil surfactant was included. In a similar lab experiment, Gannon et al. (2017) observed soil surfactant increased ^{14}C abamectin soil distribution compared to abamectin broadcasted alone.

Fungicide efficacy targeting root and crown disease are not always consistent, resulting in unacceptable turf quality and playability. The high sorption affinity of fungicides to organic matter combined with the inability of the fungicide to translocate into the roots, lends to challenges in root disease management. Previous research regarding mobility of fungicides has varied depending

on practices such as irrigation and soil surfactant use; however, further research is needed evaluating the vertical distribution of fungicides, and best management practices that aid movement. Increased fungicide movement may increase contact between active ingredient and fungicide, necessary for pathogen suppression. Mefenoxam was chosen as an ideal fungicide due to its common use on *Pythium* spp., as well as its properties that suggest moderate to high potential for soil mobility. The objective of this research was to quantify ^{14}C-mefenoxam distribution as affected by Cascade Plus, a commonly used soil surfactant, under controlled laboratory conditions.

Materials and Methods

Laboratory experiments were initiated March 20, 2018 and April 19, 2019 in Raleigh NC (NCSU Pesticide and Trace Element Lab) to evaluate the effect of a soil surfactant on ^{14}C-mefenoxam soil distribution. Polyethylene terephthalate lysimeter columns (20.6 cm length by 5.7 cm diameter) were packed with USGA specification putting green rootzone mix (90:10 sand:peat moss v:v) (Divots Inc., Sanford, NC). Inner walls of the lysimeters were coated with 1.4 mm to 2 mm coarse sand to prevent preferential flow along the edge of the lysimeter. Lysimeters were hand-packed with 150 g pea gravel (height of 4.5 cm), and rootzone mix was packed on top of this layer to a bulk density of 1.38 g cm^{-3} (depth of 15 cm). Rubber caps were placed on the bottom of the lysimeters, and six holes (5.6 mm) were drilled to allow drainage. In order to prevent soil leakage, coffee filters were placed inside of the lysimeters bottom to allow only water to drain.

Prior to any treatment, soil lysimeters were saturated four times and allowed to drain gravitationally. At 24 hrs after the last saturation, lysimeter masses were recorded to use as a measurement of moisture correction to maintain field capacity. The soil surfactant (Cacade Plus^{TM}) was syringe applied (19.1 L ha^{-1}; 5.15 μL lysimter^{-1}) to unique lysimeters. Immediately following

surfactant application, treated and non-treated lysimeters, received 0.6 cm irrigation. ^{14}C-mefenoxam was applied via pipette (763 g a.i. ha^{-1}; 0.1µCi lysimeter^{-1}) 24 hrs after the soil surfactant to the lysimeter soil surface. Immediately following treatment, a total of 0.6 cm irrigation was applied to all lysimeters.

Soil moisture was replenished to field capacity at every 48 hours based on individual lysimeter weight and leachate was collected between these days. Soil sample collection occurred 0, 3, 7, 14, and 21 DAT. Soil lysimeters were split length-wise to expose the soil profile and soil was collected from bottom to top; 4.5 cm gravel layer, 15 to 12.5 cm, 12.5 to 10 cm, 10 to 7.5 cm, 7.5 to 5 cm, 5 to 2.5 cm and 2.5 to 0 cm depths (i.e. from lowest concentration of radiolabeled material to highest) to prevent ^{14}C-mefenoxam contamination to deeper depths. Soil increments were collected and placed into 16.5 x 14.9 cm Ziplock bags. Following collection, soil was homogenized and kept frozen (-12°C) until analysis.

Sample bags were homogenized by hand-mixing for 2 min and 1 g subsamples were removed from each sampling depth and combusted for 4 min at 900°C in an OX-500 Biological Oxidizer (R.J. Harvey Instrument Corp., Tappan, NY). Molecular bonds were broken during combustion and bound to O_2 molecules to form CO_2, which was then quenched into 20 mL of scintillation cocktail (^{14}C Liquid scintillation cocktail, R.J. Harvey Instrument Corp., Tappan, NY). Scintillation vials were placed into a liquid scintillation counter (Tri-Carb 2800RT, Perkins Elmer Inc., Waltham, MA) and radioactivity was measured in disintegrations min^{-1}. Concentrations of each radiolabeled fungicide per depth were calculated based on the disintegrations min^{-1}. Data are presented as % of ^{14}C-mefenoxam recovered from the total applied.

Appropriate controls including blanks, nontreated samples, fortified samples, and control spikes were included.

Treatments were arranged in a completely randomized design with four replicates per run. Replications were unique lysimeters. Nontreated and mefenoxam- treated lysimeters without soil surfactant controls were included to validate application methods and treatment effects. Data were subjected to ANOVA (p=0.05) and means were separated using Fisher's LSD *t* test (p=0.05) using PROC MIXED (Statistical Analysis Software, Version 9.4, SAS Institute, Cary, NC).

Results

ANOVA revealed difference between 2018 and 2019 ($P < 0.0001$); therefore, data were not pooled over years. Sample depth was significant ($P < 0.0001$); therefore, data were sorted by depth. Additionally, data were pooled across sample collection timings ($P = 0.7309$). Total recoveries for the 2018 ranged from 91-107% of the applied ^{14}C mefenoxam. Across all sampling timings and treatments, greater than 90% of the recovered mefenoxam was retained in the top 2.5 cm of soil (Table 3.1). The concentration of mefenoxam in the 0 – 2.5 cm depth was not affected by soil surfactant inclusion. In the 2.5-5 cm sampling depth, differences in mefenoxam concentration as affected by soil surfactant were observed. Lysimeters treated with soil surfactant resulted in more mefenoxam (5.8% of applied) when compared to no surfactant (2.9%) in the 2.5-5 cm soil depth. Mefenoxam was not recovered below 5 cm for any treatment during any sample collection timings.

Total recoveries for the 2019 ranged from 84-103% of the total applied ^{14}C mefenoxam. Across all sampling timings and treatments, greater than 31% of the recovered mefenoxam was

retained in the top 2.5 cm of soil (Table 3.2). The concentration of mefenoxam was greater (65%) in 0-2.5 cm without soil surfactant application than with soil surfactant (32% of applied). In the 5-7.5 cm sampling depth, including a surfactant resulted in increased mefenoxam (18%) than without the use of surfactant (7% of the applied). Similarly, in the 7.5-10 cm depth, >11% more mefenoxam was detected when lysimeters received soil surfactant, than without surfactant. A similar trend was observed in the 10-12.5 cm soil depth, where >10% more of applied mefenoxam was found when lysimeters received soil surfactant prior to mefenoxam treatment. In the 12.5-15 cm depth, mefenoxam detection in surfactant treated lysimeters was increased (4.4%) compared to no surfactant (0.9% of the applied).

Discussion

Previous research has been conducted with the objective of evaluating best management practices that will promote fungicide distribution in the soil profile. The use of post application irrigation has been observed to be effective in increasing vertical fungicide movement (Starrett et al. 1996; Hutchens et al, 2019; Latin & Ou, 2018; Hockemeyer & Latin, 2015). Starett et al. (1996) concluded increased irrigation amount resulted in greater movement of metalaxyl compared to lower irrigation amount. Hutchens et al. (2019) found overall movement of myclobutanil and tebuconazole were enhanced with increased irrigation amount. Latin & Ou (2018) observed greater fungicide residue in the roots and soil of a creeping bentgrass putting green when 0.51 cm of post application irrigation was applied. Similarly, Hockemeyer & Latin (2015) found measurable amounts of fungicide were present in all turf sample components (verdure, thatch, roots, and sand) within 5 hours of application when 0.35 cm irrigation was applied immediately after fungicide application.

The use of soil surfactants in tandem with fungicide applications also been studied; however, varying results have been reported (Ou & Latin, 2018; Larsbo et al., 2008; Hutchens et al., 2020; Gannon et al., 2017). In the present study, 2018 data reported limited movement of mefenoxam, where greater than 90% of the recovered mefenoxam was retained in the top 2.5 cm of soil. In the 2.5 to 5 cm depth, 2-times more mefenoxam was recovered when treated with soil surfactant; however, this amount was only 2.9% more of applied mefenoxam. Results agree with Gardner & Branham (2001), where researchers observed that the majority of propiconazole and mefenoxam remained in the thatch layer or upper 2 cm of soil. Similarly, Larsbo et al. (2008) researched the effect of non-ionic surfactant on iprodione or azoxystrobin and propiconazole movement in lysimeters and found that in a greens-mixed (GM) soil composition, fungicide leaching was close to zero. Ou & Latin (2018) observed no effect of wetting agent revolution (Aquatrols, Paulsboro, Nj) on azoxystrobin, fluxapyroxad, propiconazole and pyraclostrobin distribution in soil and reported the majority of evaluated fungicides remained in the thatch layer. Their research supports the notion that soil surfactants may not contribute to root disease control by facilitating fungicide distribution in the turf profile.

Other research found the use of soil surfactant Qualibra (Syngenta, Greensboro, NC) increased nematicide abamectin distribution in a soil lysimeter (Gannon et al., 2017). These results are significant due to abamectins very high K_{oc} (4,000 mL g^{-1}) and low water solubility (.0078 mg L^{-1}) making its distribution unlikely in soil. Furthermore, Hutchens et al., (2020) observed soil lysimeters not treated with a soil surfactant retained at least 19.4% more myclobutanil in the top 2.5 cm of soil than when treated with a soil surfactant. Myclobutanil is reported having low mobility in soil (K_{oc} = 950 mL g^{-1} & K_s = 140 mg L^{-1}), yet pre-treated soil surfactants increased its ability to distribute. These research efforts align with data observed in 2019 of the present study,

where mefenoxam movement was enhanced through the soil lysimeter when treated with a soil surfactant. Mefenoxam concentration was 2-times higher in the top 2.5 cm without soil surfactant application than with soil surfactant. Movement of mefenoxam was detected in the 12.5 to 15 cm depth, regardless of surfactant inclusion. Authors believe that mefenoxam physicochemical properties, particularly its high-water solubility (K_s = 26 g/L), can be blamed for increased distribution compared to studies evaluating propiconazole, pyraclostrobin and myclobutanil. Additionally, we evaluated soil surfactant Cascade Plus, a non-ionic straight block co-polymer, known for enhancing water movement and leaching programs. Previous research evaluated fungicide movement as affected by soil surfactant revolution (Ou & Latin, 2015; Hutchens et al., 2020). Revolution is a modified methyl-capped block co-polymer, formulated to enhance natural capillarity balance in the root zone, allowing for vertical and lateral water movement. The features of Cascade Plus may have further contributed to mefenoxam distribution. It is observed that in 2019, mefenoxam movement was increased regardless of soil surfactant inclusion. Differences between 2018 and 2019 is speculated to be attributed to application watering technique, where increased pressure was used to syringe irrigation on bare soil lysimeter surface in 2019, compared to less pressure in 2018. Increased pressure of irrigation may have increased mefenoxam movement in the lysimeter.

Conclusions

This research was designed to investigate the effect of soil surfactant treatment on ^{14}C-mefenoxam movement through a constructed soil lysimeter, replicating the make-up of a USGA putting green rootzone complex. In both years of research, including a soil surfactant increased mefenoxam movement through the soil lysimeter. In year 1, mefenoxam vertical movement was

limited to the top 5 cm of the soil lysimeter, significantly less than observed during year 2. Research supports the notion that soil surfactant inclusion may enhance fungicide movement and delivery to pathogens in the root zone.

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Table 3.1 Treatment by sampling depth interaction on mefenoxam soil distribution.

	Depth (cm)	
Treatment[a]	0 – 2.5	2.5 – 5[b]
	----------% of applied[c] ----------	
Mefenoxam + Cascade	91.8 a[d]	5.8 a
Mefenoxam	91.6 a	2.9 b

[a] Research initiated 20 March, 2018 on USGA 90:10 (sand:peat) rootzone soil lysimeter.
[b] Mefenoxam was not detected below 5 cm.
[c] Percent of nominal 0.76 kg ai ha^{-1} application rate.
[d] Means followed by same letter within each depth are not significantly different according to Fishers Protected LSD ($P<0.05$).

Table 3.2 Treatment by sampling depth interaction on mefenoxam soil distribution.

	Depth (cm)					
Treatment [a]	0-2.5	2.5-5	5-7.5	7.5-10	10.12.5	12.5-15
	---------- % of applied [b]----------					
Mefenoxam + Cascade	31.87 b[c]	25.42 a	18.18 a	13.13 a	10.36 a	4.43 a
Mefenoxam	64.54 a	21.53 a	7.15 b	2.04 b	0.00 b	0.87 b

[a] Research initiated 19 April, 2019 on USGA 90:10 (sand:peat) rootzone soil lysimeter.
[b] Percent of nominal 0.76 kg ai ha^{-1} application rate.
[c] Means followed by same letter within each depth are not significantly different according to Fishers Protected LSD ($P<0.05$).