ABSTRACT

JEFFRIES, MATTHEW DONNEL. Off-Target Pesticide Displacement and Fate in Turfgrass, Riparian and Aquatic Systems. (Under the direction of Drs. Travis W. Gannon and Fred H. Yelverton).

Understanding pesticide environmental fate and behavior allows turfgrass managers to maximize on-target efficacy, and minimize adverse off-target effects. With this underlying premise, field and greenhouse research was conducted to characterize various environmental fate processes of 2,4-dimethylamine salt (2,4-D) and/or azoxystrobin (methyl(E)-2-{2[6- (2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate), two pesticides commonly utilized in turfgrass systems.

Field research was conducted to quantify 2,4-D and azoxystrobin persistence in clippings collected from three turfgrass species {hybrid bermudagrass (Cynodon dactylon L. x C. transvaalensis), tall fescue [Lolium arundinaceum (Schreb.) Darbysh.] or zoysiagrass (Zoysia japonica Steud.)}. 2,4-D and azoxystrobin were broadcast sprayed to unique plots at 32, 16, 8, 4, 2, 1 or 0 d before clipping collection (DBCC). Following clipping collection, pesticide residue was quantified in vegetation, and release from clippings into pond water was quantified in a subsequent greenhouse experiment. Overall, pesticide removal via clipping collection ranked hybrid bermudagrass > tall fescue > zoysiagrass, which is likely due in part to varying canopy and physiological dynamics. Pesticide detection commonly occurred in samples collected 32 DBCC, suggesting clipping management should be a long-term consideration in treated turfgrass systems. 2,4-D more readily released from clippings into water than azoxystrobin, which is likely due to the former compound being more water
soluble than the latter. Lastly, pesticide residue in water increased as the period between pesticide application and clipping collection narrowed, suggesting clippings collected sooner after application pose greater risk for adverse off-target effect(s).

Research to date has confirmed 2,4-D may dislodge from turfgrass; however, experiments have not compared turfgrass species or samples collected at various times within a day (TWD). In two field experiments, dislodgeable 2,4-D was compared between dormant hybrid bermudagrass and non-dormant perennial ryegrass (Lolium perenne L.), as well as at differing TWD (5:00, 7:00, 9:00, 11:00 or 13:00 EST) from non-dormant hybrid bermudagrass. Across experiments, dislodgeable 2,4-D was quantified at 0, 1, 2, 3, 6, 12 or 24 d after treatment (DAT). Results suggest dislodgeable 2,4-D declines as DAT increases, with non-detection consistently occurring beyond 6 DAT. Dislodgeable 2,4-D varied between turfgrass species, with greater dislodge from non-dormant perennial ryegrass than dormant hybrid bermudagrass. Additionally, dislodgeable 2,4-D decreased as TWD increased, with < 0.1% of the applied dislodged at all 13:00 sample timings. Further, increasing dislodgeable residue from 13:00 – 1 and 2 DAT to 5:00 of the subsequent DAT suggests 2,4-D re-suspended on treated turfgrass vegetation overnight, which was moderately to strongly correlated with climatic conditions favoring canopy moisture presence.

Despite concerted efforts by turfgrass managers to prevent pesticide transfer from the intended site, scenarios can unfold that lead to residue movement into off-target areas such as surface water. Therefore, greenhouse research was conducted to evaluate the 2,4-D and azoxystrobin bioremoval capacity of aquatic plants [arrow arum (Peltandra virginica L.), pickerelweed (Pontederia cordata L.) or Virginia iris (Iris virginica L.)] native to the
southeast United States. Unique plant containers were adjusted to a 5 mg L$^{-1}$ pesticide-water concentration at initiation, and water samples were collected 0, 2, 4, 7, 14 and 28 DAT for residue analysis. Results suggest aquatic plants more readily removed azoxystrobin from water than 2,4-D. This may be due in part to compromised plant growth via herbicide injury, which was observed across all species. Overall, Virginia iris reduced pesticide residue in water more than arrow arum or pickerelweed at 28 DAT.

Information from this research improves our understanding of pesticide environmental fate and behavior in clippings, dislodgement from treated vegetation, and removal from water via phytoremediation. Ultimately, this may be used to develop best management practices to prevent off-target pesticide movement via clipping displacement and human exposure via contact with treated vegetation, as well as aid with plant selection prior to establishing/renovating riparian or stormwater wetland areas that neighbor turfgrass systems.
Off-Target Pesticide Displacement and Fate in Turfgrass, Riparian and Aquatic Systems

by
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DEDICATION

This project is dedicated to my loved ones.

For it is the desire to make every one of you proud that has lead me down this road.
BIOGRAPHY

Matthew D. Jeffries was born in Alabama, and at the age of 12 he relocated with his family to Asheboro, North Carolina, where his parents still reside. Growing up, he played baseball and golf competitively for many years and during this time he routinely managed home lawns throughout the community. Upon entering the work force in high school, he found a natural fit as a golf course greenskeeper, and it was there that he developed a genuine passion for turfgrass management. The desire to gain more knowledge in this field, coupled with a draw to the outdoors lead him to pursue a B.S. degree in Turfgrass Science at North Carolina State University, which he completed with honors in 2009. Following this, he obtained a M.S. degree at North Carolina State University in Crop Science under the direction of Dr. Fred Yelverton focusing on weed management in turfgrass systems. From this great experience, lead to another, working on a Ph.D. under the direction of Drs. Travis Gannon and Fred Yelverton at North Carolina State University in the Department of Crop and Soil Sciences, researching pesticide environmental fate and behavior in turfgrass systems. Thus far, Matthew has authorship on 31 peer-reviewed publications, six extension publications, and 39 scientific abstracts, including 1st place in 2015 ASA, CSSA & SSSA graduate student oral competition. Additionally, he has taught four Weed Science courses and delivered 16 extension presentations. Awards obtained throughout his graduate career include the 2015 Turfgrass Council of North Carolina Eagle’s Award, 2016 ASA, CSSA & SSSA Future Leaders in Science Award, 2016 Weed Science Society of North Carolina Outstanding Ph.D. Student Award, 2017 Dr. James Watson SAFE Graduate Student
Fellowship and the 2017 Weed Science Society of America Outstanding Graduate Student.
Following graduation, Matthew intends to continue conducting research that benefits land managers through an improved understanding of pesticide environmental fate and behavior.
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I would like to begin by thanking my committee members, Drs. Travis Gannon, Fred Yelverton, Rick Brandenburg and Richard Cooper. Your guidance through has been instrumental in completion of this research, and I cannot thank you enough for providing an environment that allowed me to develop both personally and professionally.

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A REVIEW OF THE LITERATURE

Evaluated Pesticides

Synthetic pesticides are utilized in turfgrass systems to maintain functional, safe areas for people to utilize. As with any pesticide, various transfer and transformation processes ensue once released into the environment. The underlying premise of this research was to evaluate two pesticides widely used throughout turfgrass systems that have properties favoring the environmental fate processes of interest.

2,4-D dimethylamine salt (2,4-D) is a synthetic auxin herbicide widely used for postemergent broadleaf weed control in turfgrass systems (USEPA 2005). Registered in the United States in 1941, over 650 products containing 2,4-D were registered in over 300 distinct agricultural sites by 2005 (USEPA 2005). Specific to 2,4-D use in turfgrass, Borges et al. (2004) reported 28% of annual use in the United States (22 million kg; 1992 to 2000) was in managed turfgrass systems. Azoxystrobin (methyl(E)-2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) is a systemic strobilurin fungicide used for controlling turfgrass diseases such as brown patch (Rhizoctonia solani), fairy ring (Agrocybe pediades) and gray leaf spot (Pyricularia grisea). Registered in the United States in 1997, azoxystrobin was the world’s largest gross selling fungicide in 2008 ($900 million US) (USEPA 1997; Leadbeater 2012). Both pesticides are currently registered for use on athletic fields, commercial/residential lawns, golf courses and parks, and can be applied to most turfgrass species managed in the United States (Anonymous 2014a, 2014b).

2,4-D and azoxystrobin are classified by the United States Environmental Protection Agency as mobile and moderately mobile in soils, respectively, suggesting they can move...
from the intended site (USEPA 1997, 2005). Chemical properties relevant to 2,4-D transport from the intended site include a very high water solubility ($K_s = 796,000 \text{ mg L}^{-1}$) and a moderate soil organic carbon sorption coefficient ($K_{oc} = 20 \text{ mL g}^{-1}$), while azoxystrobin off-target transport potential is due to a moderate to long field half-life ($T_{1/2} = 72$ to $164$ d) coupled with a low affinity for sorption to coarse textured soils ($K_d = 1.5$ to $4 \text{ mL g}^{-1}$ on loamy sand and sand) (USEPA 1997, 2005). Azoxystrobin sorption affinity increases on finer textured soils ($K_d = 5$ to $23 \text{ mL g}^{-1}$), suggesting off-target transport is less likely (USEPA 1997).

2,4-D and azoxystrobin relocation from the intended site may result in off-target issues, as both compounds pose ecotoxicological concerns. Acute 2,4-D exposure is moderately toxic to freshwater fish [bluegill ($Lepomis macrochirus$) $LC_{50} = 0.9 \text{ mg L}^{-1}$ and rainbow trout ($Oncorhynchus mykiss$) $LC_{50} = 1.1 \text{ mg L}^{-1}$], and moderately to highly toxic to aquatic flora [freshwater diatom ($Navicula pelliculosa$) $EC_{50} = 3.4 \text{ mg L}^{-1}$ and parrotfeather ($Myriophyllum sibiricum$) $EC_{50} = 0.005 \text{ mg L}^{-1}$, respectively] (USDA 2006; USEPA 2016a; Walters 2004). Acute azoxystrobin is highly toxic to freshwater fish [rainbow trout $LC_{50} = 0.47 \text{ mg L}^{-1}$] and invertebrates [waterflea ($Daphnia$ spp.) $LC_{50} = 0.26 \text{ mg L}^{-1}$], as well as moderately to highly toxic to aquatic flora [duckweed ($Lemma minor$) $EC_{50} = 3.4 \text{ mg L}^{-1}$ and green algae ($Chlorophyta$ spp.) $EC_{50} = 0.4 \text{ mg L}^{-1}$, respectively] (Rodrigues et al. 2013; USEPA 1997; USEPA 2016a).

While 2,4-D and azoxystrobin have been extensively researched in many agricultural and ecological contexts, reduced efforts have been made incorporating both settings in many systems, including turfgrass. Due to the wide use of 2,4-D and azoxystrobin, coupled with
properties favoring various off-target transport routes, additional research is warranted to ensure their use in turfgrass systems does not pose unacceptable risks to neighboring ecosystems. By doing so, this information may be used to identify areas of concern that can be addressed through modifications to management practices and/or use patterns so that on a much broader scale, pesticide use in the turfgrass industry may be preserved for future practitioners.

**Pesticide Dislodge in Turfgrass Systems**

Established turfgrass canopies inherently intercept foliar-applied pesticides, which can be dislodged through various processes. Due to reported short half-lives in turfgrass vegetation ($T_{1/2} < 7$ d), pesticide dislodge from the treated area is not considered a long-term concern; however, appreciable amounts can be dislodged in the days immediately following an application (Magri and Haith 2009; Petrovic et al. 1994). Sears et al. (1987) reported wiping cheesecloth over the treated area immediately after application dislodged 10% of the applied diazinon. Similarly, Harris and Solomon (1992) conducted an experiment simulating a recreational setting and reported 8% of the applied 2,4-D was dislodged onto running shoes 1 h following application. Morgan et al. (2008) reported 2,4-D residue detection in 85% of the hand wipe samples from 135 preschool-aged children and their adult caregivers after a 48 h “observational” period at their respective homes, where participants went “about their normal, everyday lives with no added exposure or manipulation of their behavior.”

Occupational and residential pesticide exposure test guidelines currently employed by the United States Environmental Protection Agency require foliar dislodgeable residue
dissipation tests for pesticide registration and/or re-registration (USEPA 2016b). The purpose of such tests is to quantify pesticide residue remaining on treated surfaces that may be dislodged through various processes, which can lead to various forms of human exposure (USEPA 1996). Within current protocols, experiment site selection considerations only reference climatic conditions representative of the intended use area (USEPA 2012). There is no mention of turfgrass canopy conditions that may affect pesticide dislodge including acceptable cover, moisture presence or mowing practices (USEPA 2012). Thompson et al. (1984) reported mowing Kentucky bluegrass (Poa pratensis L.) 1 d after 2,4-D treatment resulted in a two-fold dislodge reduction at 2 and 3 d after treatment (DAT). Foliar dislodgeable residue dissipation protocols also do not provide recommendations on turfgrass species selection, despite previous research confirming pesticide uptake, translocation and metabolism may differ across turfgrasses with relatively comparable growth properties (ex. within cool- or warm-season turfgrasses) (Kohler and Branham 2002; McCullough et al. 2009; USEPA 2012; Yu et al. 2013).

While foliar dislodgeable residue dissipation tests are required for pesticide registration and re-registration in the United States, reports from this research pale in comparison to the amount available covering other environmental fate process such as soil degradation, leaching, runoff, etc. Furthermore, foliar dislodgeable residue reports suggest there is minimal continuity across research approaches, which limits data comparisons. Considering pesticide dislodge from treated turfgrass can serve as a direct route of human exposure, additional research is warranted to elucidate factors that affect foliar dislodgeable residue. This information will improve future foliar dislodgeable residue dissipation tests
through an improved understanding of factors researchers should consider prior to experimentation, which ultimately will ensure unacceptable risk thresholds for human-pesticide exposure in turfgrass systems are not exceeded.

**Pesticide Behavior in Turfgrass Clippings**

To maintain a healthy, functional and aesthetically pleasing surface, turfgrass systems are routinely mown during periods of active growth (Lewis et al. 2013; Christians 2011). Mowing inherently removes vegetation, commonly termed “clippings”, from the turfgrass surface. Although it is typically recommended to return clippings from mowing to the turfgrass canopy for nutrient cycling, scenarios may unfold where clippings must be collected and subsequent management practices must be performed (Bruneau et al. 2008; Lewis et al. 2013). Additionally, clipping collection is almost always required in certain settings such as golf course putting greens.

Due to legislation throughout much of the United States prohibiting clipping disposal in landfills, turfgrass managers often must find alternate ways to manage collected clippings. Such clipping management practices include composting and use for garden mulch, both environmentally friendly uses (Bahe and Peacock 1995). However, previous research has shown clippings collected from areas previously treated with a pesticide may adversely affect neighboring ecosystems via pesticide release from vegetation during decomposition (Bahe and Peacock 1995; Lewis et al. 2013; Miltner et al. 2003). Bahe and Peacock (1995) reported mulching with clippings collected from tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] 14 DAT with the synthetic auxin herbicides, 2,4-D + dicamba + mecoprop (5 kg ai
ha⁻¹), reduced cucumber (Cucumis sativus) and tomato (Lycopersicon esculentum) plant dry weight 80 and 73%, respectively, compared to plants mulched with clippings collected from nontreated plots. Lewis et al. (2013) reported clippings collected from tall fescue 14 DAT with the synthetic auxin herbicide, aminocyclopyrachlor (84 g ae ha⁻¹), controlled alligatorweed (Alternanthera philoxeroides) and parrotfeather 49 and 61%, respectively, 70 d after clipping incorporation in water. Although information from Bahe and Peacock (1995) and Lewis et al. (2013) confirming herbicide residue in turfgrass clippings may cause off-target plant injury, residue in clippings was not quantified.

Research to date quantifying pesticide residue removal via clipping collection is inconclusive. A review by Magri and Haith (2009) covering pesticide degradation in turfgrass systems states “clippings do not seem to contribute importantly to pesticide removal.” The authors expanded on this by providing evidence from laboratory research reporting < 1% removal of chlorothalonil, metalaxyl, chlorpyrifos and trichlorfon via creeping bentgrass (Agrostis stolonifera L.) clipping collection (Magri and Haith 2009; Wu et al. 2002a, 2002b). These results contrast Miltner et al. (2003), who reported 35% removal of clopyralid, a synthetic auxin herbicide, from 10 mowing events over 70 DAT. Additionally, the authors reported ecotoxicologically concerning concentrations (900 µg kg⁻¹; susceptible plant activity ≈ 5 µg kg⁻¹) in cool-season turfgrass clippings collected 70 DAT (Miltner et al. 2003).

While research has confirmed pesticide residue may be removed from turfgrass systems via clipping collection, minimal efforts have been made to put these results into context as a proportion of the field application. Furthermore, select cases where this was
conducted produced conflicting results, which is likely due in part to differing field conditions and pesticide physicochemical properties. This suggests additional pesticides and geographies should be evaluated to more holistically understand this environmental fate process in turfgrass systems. By doing so, turfgrass managers will be able to improve management practices by maximizing on-target pesticide efficacy, and minimizing adverse off-target effect via clipping displacement.

**Plant Selection to Optimize Pesticide Phytoremediation**

Despite concerted efforts by turfgrass managers to prevent pesticide off-target transport, scenarios can unfold where compounds relocate to unintended areas via drift, runoff, etc. (Nett et al. 2008). A notable pesticide sink from off-target transport are surface water bodies, which was highlighted in a 2004 national scale survey of surface water pesticide contamination that found ≥ 1 pesticide/metabolites in > 90% of the 186 test sites (USGS 2006). The report highlighted aquatic ecosystem concerns in urban areas, as detection of pesticide exceedances of an aquatic life benchmark was ≈ 25% greater in this land use than agricultural, undeveloped or mixed-use sites (USGS 2006).

Phytoremediation is the use of plants and their associated microbes for environmental decontamination (Pilon-Smits 2005). Constructed stormwater wetlands are commonly established in areas neighboring turfgrass systems for phytoremediation, as well as to provide wildlife habitat (Hunt and Doll 2000; Libby and Harker 2004; NCDENR 2007). Design and management specifications for these systems are immense, and beyond the scope of this review; however, it should be noted that despite previous reports of bioremoval capacity
commonly differing between plant species in these systems, plant selection recommendations commonly do not incorporate this information for in situ practice (Hunt and Doll 2000; Libby and Harker 2004). Rice et al. (1997) reported hornwort (*Ceratophyllum demersum*), American elodea (*Elodea canadensis*) and common duckweed all reduced metolachlor and atrazine, two herbicides, residues in water (38 to 60% less residue than non-planted containers). However, hornwort and American elodea reduced residue in water (1 to 4% of applied remaining at 16 DAT) more than common duckweed (23% of applied remaining at 16 DAT) (Rice et al. 1997). Interestingly, from a separate experiment, common duckweed was the best suited plant species [compared to Canadian waterweed (*Elodea canadensis*) and Carolina fanwort (*Cabomba aquatica*)] for removing residue of the pesticides, copper sulfate, flazasulfuron and dimethomorph, from water (Olette et al. 2008).

Plant selection considerations for constructed stormwater wetlands typically focus on promoting diversity, and selecting non-invasive, native species that are well adapted for the specific growth setting of interest (Hunt and Doll 2000; NRCS 2000). While these are sound principles, plant selection for mitigation areas that neighbor turfgrass systems may pose unique challenges. For example, an upright plant growth habit may be undesirable on golf courses due to playability issues (Bass et al. 2012). Additionally, plants that pose allergic and tactile injury issues are not advisable for public health concerns (Libby et al. 2004). With these considerations in mind, Smith et al. (2008) evaluated riparian plant species to optimize phytoremediation of pesticide off-target transport from treated turfgrass in the northeast United States. The authors reported blue flag iris (*Iris versicolor*), eastern gama grass (*Tripsacum dactyloides*) and big blue stem (*Andropogon gerardii*) were best suited to
remove chlorpyrifos (47 to 76% removal), chlorothalonil (91 to 95%), pendimethalin (17 to 48%) and propiconazole (22 to 33%) from soil over 7 mo (Smith et al. 2008).

While previous research has shown plant-pollutant bioremoval capacity may differ across species and/or compounds, this premise has been minimally investigated with plants suitable for establishment in stormwater wetlands neighboring turfgrass systems. Furthermore, species adapted to environmental conditions common to the southeast United States have not been investigated. Therefore, research is warranted to identify plant species native to the southeast United States best suited to mitigated pesticide off-target transport from turfgrass systems.
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CHAPTER 1: 2,4-D AND AZOXYSTROBIN PERSISTENCE IN AND RELEASE FROM TURFGRASS CLIPPINGS.

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Previous research has shown pesticide residue within clippings from previously treated turfgrass may become bioavailable as they decompose, adversely affecting off-target organisms. Field research was conducted to quantify 2,4-D and azoxystrobin residues in turfgrass clippings collected from hybrid bermudagrass [Cynodon dactylon (L.) Pers. × C. transvaalensis Burtt-Davy], tall fescue [Lolium arundinaceum (Schreb.) S.J. Darbyshire], and zoysiagrass (Zoysia japonica Steud.). A subsequent greenhouse experiment was conducted to measure pesticide release from clippings into water. 2,4-D (1.6 kg ai ha⁻¹) and azoxystrobin (0.6 kg ai ha⁻¹) were applied to unique plots at 32, 16, 8, 4, 2, 1, or 0 d before clipping collection (DBCC). Clippings were collected from each experimental unit for pesticide residue analysis and to quantify pesticide release from clippings into water. 2,4-D and azoxystrobin were both detected when turfgrass was treated from 0 to 32 DBCC, suggesting clipping management should be implemented for an extended period of time following application. Pesticide residue was detected in all water samples collected, confirming 2,4-D and azoxystrobin release from turfgrass clippings; however, release varied between compounds. Two d after clippings were incorporated in water, 39 and 10% of 2,4-D
and azoxystrobin released from clippings collected 2 DBCC, respectively. This research improves our knowledge of behavior/persistence of two commonly applied pesticides in three turfgrass species widely utilized in the United States, which can be used to develop best management practices to reduce potential adverse off-target environmental impacts associated with pesticide use in turfgrass systems.

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Introduction

Turfgrasses are grown on over 16.3 million ha in the contiguous United States (US) – exceeding the combined area of irrigated grain corn ([Zea mays L.] 2.5 million ha), soybeans ([Glycine max L.] 2.1 million ha), and cotton ([Gossypium hirsutum L.] 0.9 million ha) – and are commonly treated with pesticides to maintain acceptable stand quality (Milesi, 2005; USDA, 2009). The US Environmental Protection Agency reported in 2011 that 30 million kg synthetic pesticides were applied to homes and gardens from 2006 to 2007, which accounted for 8% of total US pesticide use (Grube et al., 2011). Various pesticide transport processes may ensue following an application to turfgrass, with one being movement from the intended site via turfgrass clippings collected from a previously treated area (Magri and Haith, 2009).

A standard cultural practice in managed turfgrass systems is routinely mowing during periods of active growth to maintain a healthy, functional, and aesthetically pleasing surface (Lewis et al., 2013; Christians, 2011). This practice inherently removes vegetation, commonly termed “clippings”, from the turfgrass surface. While it is typically recommended to return clippings from mowing to the turfgrass canopy, scenarios may unfold where clippings must be collected and subsequent management practices must be performed (Bruneau et al., 2008; Lewis et al., 2013). Additionally, turfgrass clipping collection is still a common practice in residential sites. Due to legislation in most US states prohibiting clipping disposal in landfills, turfgrass managers often must find alternative ways to manage collected clippings. Common clipping management practices include composting and use for garden mulch due to clipping’s high moisture and nutrient content (Bahe and Peacock, 1995).
While these are environmentally-friendly clipping uses, previous research has shown clippings collected from areas previously treated with a pesticide may cause adverse off-target impacts to aquatic or terrestrial organisms via pesticide release from vegetation during decomposition and subsequent uptake at injurious levels (Bahe and Peacock, 1995; Lewis et al., 2013; Miltner et al., 2003). Bahe and Peacock (1995) reported mulching with clippings collected from tall fescue \( \text{Lolium arundinaceum} \) (Schreb.) S.J. Darbyshire treated with 2,4-D + dicamba + mecoprop \( (5 \text{ kg ai ha}^{-1}) \) reduced cucumber \( \text{Cucumis sativus} \) L.) and tomato \( \text{(Lycopersicon esculentum} \) L.) plant dry weight 80 and 73%, respectively, compared to plants mulched with clippings collected from nontreated plots. Miltner et al. (2003) reported clopyralid \( (280 \text{ g ha}^{-1}) \), a synthetic auxin herbicide, was detected at ecotoxicologically concerning concentrations \( (900 \mu\text{g kg}^{-1}; \text{susceptible plant activity} \approx 5 \mu\text{g kg}^{-1}) \) in cool-season turfgrass clippings collected 10 wk after treatment (WAT). Lewis et al. (2013) reported clippings collected from tall fescue 2 WAT with the synthetic auxin herbicide, aminocyclopyrachlor \( (84 \text{ g ha}^{-1}) \), controlled alligatorweed \( \text{Alternanthera philoxeroides} \) (Mart.) Griseb.] and parrotfeather \( \text{Myriophyllum aquaticum} \) (Vell.) Verdc.] 49 and 61%, respectively, 70 d after clipping incorporation in water.

2,4-D dimethylamine salt (2,4-D) is a synthetic auxin herbicide commonly used for postemergent broadleaf weed control in many crops, including turfgrass (EPA, 2005). In 2005, over 650 products containing 2,4-D were registered in the US in over 300 distinct use sites. At that time, it was reported 7.3 million kg 2,4-D (34% of total US use) were applied to non-cropland areas, including turfgrass (EPA, 2005). Azoxystrobin (methyl(E)-2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) is a systemic strobilurin
fungicide used for controlling turfgrass diseases such as brown patch (*Rhizoctonia solani*), fairy ring (*Agrocybe pediades*), and gray leaf spot (*Pyricularia grisea*) (Anonymous, 2014b). Although sales were not specifically for turfgrass, azoxystrobin was the world’s largest gross selling fungicide in 2008 ($900 million US) (Leadbeater, 2012). Both 2,4-D and azoxystrobin are currently registered for use in athletic fields, commercial/residential lawns, golf courses, and parks, and can be applied to most turfgrass species managed in the US (Anonymous, 2014a, 2014b). Due to 2,4-D and azoxystrobin both having foliar and root uptake pathways coupled with being acropetally translocated in plants, they are likely to accumulate in the upper region of treated turfgrass (clippings) that is removed via mowing (Table 1; EPA, 1997, 2005). The differing water solubility suggest 2,4-D within foliage is more likely to release into water than azoxystrobin; however, azoxystrobin is stable to hydrolysis and both compound’s aqueous photolysis half-life is > 10 d (EPA, 1997, 2005). This is potentially ecotoxicologically concerning, as both pesticides are highly toxic to various aquatic organisms (EPA, 1997, 2005).

Hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. *x C. transvaalensis* Burtt-Davy] and zoysiagrass (*Zoysia* spp.) are warm-season, mat–forming turfgrass species adapted to tropical and subtropical US climatic zones (Christians, 2011). Tall fescue is a cool-season, bunch-type turfgrass that is adapted to warm temperate and cool subtropical climates (Christians, 2011). All three turfgrass species are commonly established in their respective adapted zones on commercial/residential properties, golf courses, and parks. Within North Carolina, a statewide turfgrass species composition survey determined bermudagrass, tall fescue and zoysiagrass comprised 55, 65, 83, and 79% of the known area species established
in commercial properties, single-family dwellings, golf courses, and parks (non-athletic), respectively (NCDA, 1999).

Previous research has shown grass species with relatively similar growth characteristics differentially absorb, uptake, and metabolize herbicides (Yu et al., 2013; Olson et al., 2000). Additionally, previous research has shown pesticide release from plant vegetation is influenced by compound-specific properties (Buyskonsmez et al., 1999). Research to date has not been published coupling these concepts by evaluating the persistence of multiple pesticides in clippings collected from multiple turfgrass species, as well as pesticide release from clippings during decomposition. This information will improve best management practices regarding mowing and turfgrass selection, as well as pesticide application scheduling and post-application clipping management practices. The objectives of this research were to quantify 2,4-D and azoxystrobin persistence in hybrid bermudagrass, tall fescue, and zoysiagrass clippings, as well as release from clipping vegetation into water.

**Materials and Methods**

**Research Overview**

Two experiments were conducted to complete the research objectives. The first experiment was conducted in the field, and quantified pesticide residue in clippings from various turfgrass species collected at various timings. 2,4-D and azoxystrobin were applied at various times prior to clipping collection and residues levels were quantified. A subsequent greenhouse experiment was conducted to measure pesticide release from
clippings into water. To do so, subsamples of harvested field clippings were placed into containers with pond water and subsequent water samples were collected over time.

**Pesticide Persistence in Clippings**

Field research was conducted (Lake Wheeler Turfgrass Field Lab, Raleigh, NC) to quantify 2,4-D and azoxystrobin residues in clippings from three turfgrass species collected at varying times after application. Research was conducted on a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludult) soil with pH 6.4 and 1.9% w w⁻¹ organic matter. 2,4-D and azoxystrobin were 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 2,4-D and azoxystrobin residues were non-detectable.

Evaluated turfgrass species included ‘Tifway 419’ hybrid bermudagrass, ‘K-31’ tall fescue, and ‘El Toro’ zoysiagrass. Experimental areas were maintained in accordance with current residential lawn recommendations with respect to fertility (bermudagrass = 150 kg N ha⁻¹ yr⁻¹; tall fescue and zoysiagrass = 100 kg N ha⁻¹ yr⁻¹), irrigation (provided to supplement rainfall), and mowing height (bermudagrass and zoysiagrass = 5 cm; tall fescue = 9 cm) (Bruneau et al., 2008). Pesticides or plant growth regulators were not applied to experimental areas during the research period.

2,4-D (Amine 400 2,4-D Weed Killer®, PBI Gordon Corporation, Kansas City, MO) and azoxystrobin (Heritage TL®, Syngenta Crop Protection, Incorporated, Greensboro, NC) were applied as a broadcast spray at 1.6 and 0.6 kg ai ha⁻¹ (maximum labeled single application rate), respectively, to unique 1.2 by 3 m plots (Anonymous, 2014a, 2014b). Applications were initiated 5 Aug. 2013 and completed 6 Sept. 2013. The evaluated
application timings included 32, 16, 8, 4, 2, 1, or 0 d before clipping collection (DBCC). Treatments were made with a CO₂-propelled boom comprised of three flat-fan nozzles (TeeJet 8002 XR VS Flat-Fan®, Spraying Systems Company, Wheaton, IL) calibrated to deliver 812 L ha⁻¹ (minimum labeled carrier volume across both pesticides) at 179 kPa (Anonymous, 2014a, 2014b).

From 32 to 5 DBCC, plots were mown twice weekly and clippings were returned to the stand with a self-propelled rotary mower (Honda HRC 2163HXA®, American Honda Power Equipment Division, Alpharetta, GA). The mower was sterilized with an ammonia:water (2:1 by vol) solution and dried with an air compressor between mowing in treated plots. Plots were not mown following 4 DBCC application, and clippings were harvested from all plots following the 0 DBCC application. At 0 DBCC, pesticide treatments were applied and allowed to dry for 2 h before clipping collection occurred. All applications were made to dry turfgrass vegetation from 12:00:00 to 15:00:00 Eastern Standard Time. Mowing height throughout the trial period followed the recommended “1/3 rule”, where the mower deck was set to remove 1/3 of the aboveground vegetation height (Christians, 2011). Bermudagrass, tall fescue, and zoysiagrass mowing heights at the 0 DBCC clipping collection were 5, 8, and 5 cm, respectively. Clippings were collected from each experimental unit in the rotary mower bag lined with a plastic bag (49 L; HDX Drawstring Kitchen Bags, Home Depot, Atlanta, GA), fresh mass was recorded (g), and subsampled to quantify pesticide residue with high performance liquid chromatography–diode array detector (HPLC–DAD) methodology. The bulk sample was stored at -14° C until analysis. Turfgrass clipping pesticide residue were converted to a percent of the applied:
Eq. [1] % of applied = \{[(RA 5 g^{-1} \times HM g cm^{-2}) / AR] \times 100\}

where RA, HM and AR represent residue analysis, fresh mass and application rate, respectively.

**Pesticide Release from Clippings**

Following turfgrass clipping collection in the field, a greenhouse (Method Road Greenhouses, Raleigh, NC) experiment was conducted to evaluate pesticide release from previously treated turfgrass clippings. Pond water was collected from a local source with nondetectable 2,4-D and azoxystrobin residues. At experiment initiation, 20 g fresh clippings from 2, 4, and 8 DBCC collection timings were mixed with 150 mL water in unique amber high-density polyethylene containers (500 cm³; Fisher Scientific International, Incorporated, Hampton, NH) sealed to minimize evaporative losses. Containers were stored in the dark at 25° C, hand-shaken (30 s), and opened (30 min) twice daily. Additionally, containers were re-randomized daily to minimize greenhouse microclimate effects. Check containers were included to measure 2,4-D and azoxystrobin breakdown in pond water alone, as well as to adjust for evaporative losses.

Water samples (15 mL) were collected 2 d after clipping incorporation in water and pesticide residue was determined with HPLC-DAD methods. Pesticide residue in water was converted to a percent released from the total originally in/on clippings:

Eq. [2] % release = \{[(WS \mu g) / CI \mu g] \times 100\}
where WS and CI represent water sample residue mass and total residue mass applied to container via clipping incorporation, respectively.

**Residue Analyses**

All reagents and solvents used for residue analyses were liquid chromatography–mass spectrometry grade. 2,4-D residue quantification for turfgrass clippings (all species) and water were conducted via modifications to methods by Shin et al. (2011) and Park et al. (2011), respectively. Azoxystrobin residue quantification for turfgrass vegetation (all species) and water were conducted via modifications to methods by Sundravadana et al. (2008) and EPA MRID No. 436781-89 (1993), respectively.

Modifications to Shin et al. (2011) for 2,4-D vegetation extraction included: 5 g vegetation placed in a glass jar (250 mL) and mixed with water (120 mL) in a high-speed homogenizer (9,000 rpm) for 2 min. Ten min after mixing, an aliquot (10 mL) was centrifuged (3,500 rpm; 10 min) and a sub-aliquot (7 mL) was added to sodium chloride (1 g) and partitioned with n-hexane (4 mL). The upper hexane layer was discarded and partition step repeated. The aqueous solution was acidified with 10% sulfuric acid (0.5 mL) to pH 2. The acidified aqueous solution was partitioned twice with dichloromethane (6 mL) and evaporated to dryness with N-evap (N-EVAP 112, Organomation Associated Incorporated, Berlin, MA). Samples were reconstituted in water:acetonitrile [7 mL (9:1 by vol)], sonicated (3 min), vortexed (3 min), filtered (0.45 µm nylon filter), and vialled for injection.

Modifications to Park et al. (2011) for 2,4-D water extraction included: sample size (15 mL), centrifugation duration (15 min), and two filtrations (0.45 µm nylon filter). Sample cleanup modifications included SPE cartridges (3 cm³, 60 mg; Oasis HLB Cartridges, Waters
Corporation, Milford, MA) with ethyl acetate (10 mL) as eluting solvent. Ethyl acetate was evaporated to dryness using N-evap, reconstituted in acetonitrile, diluted with water (1:1 by vol), filtered, and vialled for injection. All samples were injected within 4 h of extraction and cleanup.

Modifications to Sundravadana et al. (2008) for azoxystrobin vegetation extraction included: 5 g vegetation mixed with ethyl acetate (120 mL) in a glass jar (250 mL), supernatant (15 mL) evaporated to dryness and reconstituted in acetonitrile (1 mL). Samples where then dispensed into Quechers tubes (Q-sep® QuEChERS dSPE tubes, Catalog No. 26123, Restek Corporation, Bellefonte, PA), vortexed (2 min), centrifuged (10 min), filtered (0.45 µm polytetrafluoroethylene filter), and vialled for injection. Modifications to EPA MRID No. 436871-89 for azoxystrobin water extraction included sample size (15 mL) and centrifuge duration (15 min). Sample cleanup modifications included SPE cartridges (3 cm³, 30 mg; Strata-X 33u Polymeric Reversed Phase Cartridges, Phenomenex, Torrance, CA) with ethyl acetate (10 mL) as eluting solvent. Ethyl acetate was evaporated to dryness using N-evap, reconstituted in acetonitrile, diluted with water (1:1 by vol), filtered, and vialled for injection.

2,4–D and azoxystrobin residues were quantified for both matrices by HPLC-DAD (Agilent–1260 Infinity, Agilent Technologies, Incorporated, Wilmington, DE) equipped with a C₁₈ silica column [75 mm length x 4.6 mm i.d. (Poroschell 120EC-C₁₈; Agilent Technologies, Incorporated, Wilmington, DE)]. High performance liquid chromatography parameters for 2,4-D were 32° C column temperature; {[acetonitrile:water] 3:2} + 0.1% formic acid by vol} mobile phase; 1 mL min⁻¹ flow rate; 10 µL injection volume, and a 1.2
min retention time at 230 nm. High performance liquid chromatography 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. 2,4-D and azoxystrobin limit of detection was 0.3 and 0.05 mg L⁻¹, respectively, and limit of quantification was 1.0 and 0.25 mg L⁻¹, respectively, based on a 3:1 signal to noise ratio. Pesticide residue was quantified using peak area measurements (Open LAB CDS ChemStation, Version C.01.04, Agilent Technologies, Incorporated, Wilmington, DE). 2,4-D fortification recovery checks for all vegetation and water matrices ranged from 85 to 97% and 98 to 102%, respectively, and azoxystrobin fortification checks ranged from 91 to 98% and 97 to 103%, respectively.

**Experimental Design**

Experiments evaluated three replications of a 3-by-2-by-7 factorial treatment arrangement. Factorial levels included three turfgrass species (hybrid bermudagrass, tall fescue, or zoysiagrass), two pesticides (2,4-D or azoxystrobin), and seven clipping collection timings (32, 16, 8, 4, 2, 1, or 0 DBCC). The field experimental design was a split plot-randomized complete block with turfgrass species as main plots, and pesticide-DBCC combination subplots. The greenhouse experimental design was a randomized complete block. Both experiments included nontreated checks to ensure experimental areas were not contaminated.

**Statistical Analysis**

Statistical analyses were conducted by ANOVA (P = 0.05) using general linear models in SAS (Statistical Analysis Software®, Version 9.2, SAS Institute, Incorporated,
Cary, NC). Pesticide, turfgrass species, and DBCC were considered fixed effects. Main effects and their interactions are presented accordingly, with precedent given to significant interactions of increasing magnitude (Steel et al., 1997). Means were separated according to Fisher’s protected LSD (P < 0.05).

Results

Pesticide Retention in Turfgrass Clippings

Analysis of variance revealed significant application timing-by-turfgrass species interactions for 2,4-D data detailing clipping residue and removal from the system via clipping collection. The simple effects of application timing and turfgrass species are discussed in this order. At 0 DBCC, 2,4-D retention in clippings did not vary between turfgrass species, which is likely due to the short period of time between application and clipping collection (2 h; Table 2). At 1 and 2 DBCC, 2,4-D residue ranked tall fescue (320 and 294 mg kg\(^{-1}\), respectively) > zoysiagrass (303 and 265 mg kg\(^{-1}\)) > hybrid bermudagrass (247 and 182 mg kg\(^{-1}\)). 2,4-D retention in clippings did not vary at 4 and 8 DBCC, with residue ranging from 33 to 69 mg kg\(^{-1}\) across timing and species. At 16 and 32 DBCC, 2,4-D retention in clippings varied, with lower residue in zoysiagrass clippings (18 and 7 mg kg\(^{-1}\), respectively) than hybrid bermudagrass (25 and 21 mg kg\(^{-1}\)), or tall fescue (28 and 17 mg kg\(^{-1}\)). Within turfgrass species, 2,4-D retention in clippings declined across all species as time between application and clipping collection increased. 2,4-D residue dissipation in hybrid bermudagrass clippings outranked tall fescue and zoysiagrass, as 52, 24, and 38% of the 0 DBCC residue was present at 2 DBCC, respectively. Beyond 4 DBCC, 2,4-D residue in
clippings only varied in zoysiagrass with decreases at each sample collection timing. At 32 DBCC, 2,4-D residue was detected in clippings collected from all turfgrass species.

While minimal differences were detected between turfgrass species in plant 2,4-D residue data at 0 DBCC, 2,4-D removal via clipping collection suggests off-target transport potential differs between species. Excluding 2 and 4 DBCC application timings, 2,4-D removal via clipping collection ranked hybrid bermudagrass > tall fescue > zoysiagrass (Table 2). Within turfgrass species, 2,4-D removal via clipping collection data generally aligns with clipping residue trends. Across all species, clipping collection at 2 DBCC, which is the currently recommended period between application and mowing, removed 16.9 to 24.9% of the applied 2,4-D (Anonymous, 2014a). From 4 to 32 DBCC, 2,4-D removed via clipping collection declined; however, persistence varied between species. 2,4-D removed via hybrid bermudagrass clipping collection did not vary from 4 DBCC (5% of applied) to 32 DBCC (2.4%), while tall fescue behaved similarly from 8 DBCC (2.8%) to 32 DBCC (1.4%). 2,4-D removed via zoysiagrass clipping collection consistently declined at each sample collection timing from 4 DBCC (4.4% of applied) to 32 DBCC (0.5%). Across all species at 32 DBCC, clipping collection removed 0.5 to 2.4% of the applied 2,4-D.

Analysis of variance revealed significant application timing-by-turfgrass species interactions for azoxystrobin clipping retention data detailing plant residue and removal from the system via clipping collection. As with 2,4-D, the simple effects of application timing and turfgrass species are discussed in this order. Azoxystrobin retention in clippings did not vary between turfgrass species from 0 to 2 DBCC, with residue ranging from 44 to 81 mg kg$^{-1}$ (Table 3). At 4 and 8 DBCC, azoxystrobin retention differed, with residue ranking tall
fescue (34 and 18 mg kg\(^{-1}\), respectively) > hybrid bermudagrass (16 and 9 mg kg\(^{-1}\)) = zoysiagrass (17 and 12 mg kg\(^{-1}\)). Azoxystrobin retention in clippings did not vary at 16 and 32 DBCC, with detection occurring for all species at 16 DBCC (6 to 9 mg kg\(^{-1}\)), as well as hybrid bermudagrass (1 mg kg\(^{-1}\)) and tall fescue (1 mg kg\(^{-1}\)) at 32 DBCC. Within all turfgrass species, azoxystrobin retention in clippings generally declined as time between application and clipping collection increased. Azoxystrobin residue dissipation in hybrid bermudagrass and zoysiagrass clippings outranked tall fescue, as 30, 15, and 33\% of the 0 DBCC residue was present at 2 DBCC, respectively. Beyond 4 DBCC, azoxystrobin residue retention in clippings did not vary in hybrid bermudagrass, while gradual reductions in tall fescue and zoysiagrass clippings were observed. At 32 DBCC, 2,4-D residue was not detected in zoysiagrass clippings collected.

As with 2,4-D, azoxystrobin removal via clipping collection suggests off-target transport potential differs between species. Similar to 2,4-D, clipping collection removed more of the applied azoxystrobin from hybrid bermudagrass (30.1\% of applied) than tall fescue (20\%) or zoysiagrass (17.2\%) at 0 DBCC (Table 3). This further supports turfgrass canopy spray interception varied between species. Currently, post-application mowing interval is not specified on the azoxystrobin label; however, 11.5 to 21.2\% of the applied was removed across all turfgrass species at 2 DBCC. Beyond 2 DBCC, greatest and least azoxystrobin removal from clipping collection occurred from tall fescue and zoysiagrass, respectively, when statistical separation occurred. Within turfgrass species, azoxystrobin removal via clipping collection data generally aligns with clipping residue trends. Across all turfgrass species, maximum azoxystrobin removal from clipping collection occurred at 0
DBCC (17.2 to 30.1% of applied), while removal generally did not differ between 1 and 2 DBCC. From 4 to 32 DBCC, azoxystrobin removed via clipping collection declined; however, persistence varied between species. Azoxystrobin removed via hybrid bermudagrass clipping collection did not vary from 8 DBCC (3.1% of applied) to 32 DBCC (0.2%), while tall fescue declined at each sample collection timing from 4 DBCC (9.6%) to 32 DBCC (0.3%). Azoxystrobin removed via zoysiagrass clipping collection declined from 4 DBCC (3.5% of applied) to 16 DBCC (1.5%), and was non-detectable at 32 DBCC.

**Pesticide Release from Turfgrass Clippings**

Analysis of variance revealed a significant application timing-by-turfgrass species interaction for 2,4-D clipping data detailing residue in water and release from clippings into water. Within DBCC timings, 2,4-D residue in water followed similar trends to clipping residue. Specifically, 2,4-D residue in 2 DBCC clippings aligned with residue in water ranking tall fescue (19.1 mg L\(^{-1}\)) > zoysiagrass (17.0 mg L\(^{-1}\)) > hybrid bermudagrass (10.0 mg L\(^{-1}\); Table 4). 4 DBCC residue in water ranked zoysiagrass (7.4 mg L\(^{-1}\)) > tall fescue (3.6 mg L\(^{-1}\)) = hybrid bermudagrass (2.2 mg L\(^{-1}\)), while no differences were detected between turfgrass from 8 DBCC clippings (1.5 to 3.3 mg L\(^{-1}\)). Within turfgrass species, 2,4-D residue in water declined as DBCC increasing. 2 DBCC clippings consistently caused higher residue in water (10.0 to 19.1 mg L\(^{-1}\)) than 4 or 8 DBCC clippings. 2,4-D residue in water did not vary between 4 and 8 DBCC after hybrid bermudagrass (1.5 to 2.2 mg L\(^{-1}\)) and tall fescue (2.6 to 3.6 mg L\(^{-1}\)) clippings were introduced into water, while zoysiagrass declined from 4 DBCC (7.4 mg L\(^{-1}\)) to 8 DBCC (3.3 mg L\(^{-1}\)).
Coupling 2,4-D clipping and residue in water data, release reported as a percent of the total amount originally loaded into water from vegetation suggests turfgrass species differentially release 2,4-D. Within DBCC timings, 2,4-D release did not vary across turfgrass species from 2 DBCC clippings, while less released from hybrid bermudagrass clippings collected 4 and 8 DBCC (31 and 29% release, respectively) than tall fescue (45 and 50%) and zoysiagrass (61 and 60%; Table 4). Within turfgrass species, 2,4-D release did not vary from 2 to 8 DBCC clippings collected from hybrid bermudagrass (29 to 35% release) or tall fescue (41 to 50%), while release increased in zoysiagrass clippings collected 2 DBCC (41%) to 4 and 8 DBCC (61 and 60%, respectively).

Analysis of variance revealed a significant main effect of application timing on azoxystrobin clipping data detailing residue in water. As with 2,4-D residue in water trends, azoxystrobin residue in water decreased as DBCC increased. Averaged over turfgrass species, 2 DBCC caused greater azoxystrobin residue in water (0.91 mg L\(^{-1}\)) than 4 (0.50 mg L\(^{-1}\)) or 8 (0.29 mg L\(^{-1}\)) DBCC clippings (Table 4), which proportionally aligns with initial residue in clippings (Table 2). This is supported by no significant differences detected in azoxystrobin release data, with release ranging from 10.1 to 15.2%.

**Discussion**

**Pesticide Retention in Turfgrass Clippings**

Data from this research support the commonly recommended turfgrass management practice of returning clippings from mowing to the canopy (Bruneau et al., 2008). Aside from previous research determining returning clippings cycles essential plant nutrients (Liu
and Hull, 2006; Sartain, 1993) and increases turfgrass system carbon sequestration capacity (Qian et al., 2003), this research supports it reduces off-target pesticide transport potential from previously treated areas. At 32 DBCC, 2,4-D was detected across all evaluated turfgrass species, while azoxystrobin was detected in hybrid bermudagrass and tall fescue clippings. Detection of 2,4-D in clippings at later timings agrees with previous research evaluating 2,4-D dichlorophenoxyacetic acid metabolism in ironweed (Vernonia baldwinii Torr.), which recovered 38.4% of the initial at 3 d after treatment (DAT) and 2% at 21 DAT (Linscott and McCarty, 1962). Previous research has shown azoxystrobin persists in treated plants for an extended period after application, with 36 to 72% of total azoxystrobin detected in rice (Oryza sativa L.) grain 75 to 95 DAT remaining as the parent compound (Mastovska, 2008). Similar findings were reported in wheat (Triticum aestivum L.) forage, with 55 to 65% of total azoxystrobin remaining as the parent compound 61 to 62 DAT (Mastovska, 2008).

Differing 2,4-D and azoxystrobin removal via clipping collection at 0 DBCC suggests turfgrass canopy spray interception varied between species, as the finest textured of the three evaluated species, hybrid bermudagrass, likely retained a greater proportion of the spray solution in the upper region of the canopy that was harvested via mowing (Christians, 2011). Additional research efforts sampling the soil surface and remaining turfgrass vegetation following mowing are required to confirm this hypothesis. The significant decline from 2 to 4 DBCC in 2,4-D and azoxystrobin residues in clippings and removal via mowing is likely due to a rainfall event (7.5 mm H$_2$O) 16 h following the 4 DBCC application. Previous research has shown irrigation or precipitation may reduce 2,4-D residue in/on turfgrass
vegetation (Thompson et al., 1984). Thompson et al. (1984) reported dislodgeable 2,4-D residue declined to 0.1% of the applied 1 d after treatment following rainfall (18 mm H₂O) the evening after application. This was attributed to 2,4-D being washed into turfgrass thatch or soil, making it less dislodgeable (Thompson et al., 1984). Overall, 2,4-D and azoxystrobin dissipated slower in tall fescue than hybrid bermudagrass or zoysiagrass clippings, which is likely due in part to differing growth characteristics between species coupled with the seasonal application timings. Specifically, pesticide applications in the presented research were made mid-summer when tall fescue, a C₃-grass, is growing in suboptimal conditions that may reduce pesticide metabolism (McCullough and Hart, 2006).

The detection of 2,4-D and azoxystrobin residues in clippings at 32 DBCC suggests management practices should be implemented for at least this duration following application. Previous turfgrass research has shown herbicides and fungicides may persist in clippings similarly to 2,4-D and azoxystrobin in this research. Mahoney et al. (2015) reported clipping management practices for a 30 d post-application period should be implemented to minimize adverse effects from hybrid bermudagrass clippings produced following monosodium methylarsenate application (2.25 + 2.25 kg ai ha⁻¹ on 7 d interval), as maximum arsenic increases in clipping vegetation occurred 30 d after initial treatment. Lewis et al. (2014) reported clippings collected from tall fescue treated with aminocyclopyrachlor (79 g ae ha⁻¹) 14 to 1.75 DBCC controlled white clover (Trifolium repens L. ‘Dutch’) 73 to 92% 8 wk after clippings were applied to the stand. Miltner et al. (2003) reported turfgrass clippings should not be collected for livestock feed for 1 yr following clopyralid application. Although reports on this topic specific to fungicide persistence is limited, Frederick et al. (1994)
reported > 25% of the initial concentration of chloroneb and triadimefon, turfgrass fungicides moderately toxic to aquatic organisms, remained in Kentucky bluegrass (*Poa pratensis* L.) 3 and 8 WAT, respectively (Marrs and Ballantyne, 2004).

**Pesticide Release from Turfgrass Clippings**

2,4-D and azoxystrobin release from turfgrass clippings into water is an off-target transport process that has not previously been documented. Furthermore, limited research on this process has been reported outside of these compounds, which should be addressed. The high proportion of turfgrass clipping vegetation to pond water required for pesticide residue detection limits the implications of the results to in situ settings; however, it can concluded that 2,4-D and azoxystrobin release from hybrid bermudagrass, tall fescue, and zoysiagrass clippings treated 2, 4, and 8 DBCC. Additionally, release varied between compounds, with increasing water solubility likely causing greater releasal. Averaged over turfgrass species, 39, 46, and 46% of 2,4-D released from clippings collected 2, 4, and 8 DBCC, respectively, while 10, 15, and 15% released from the less water soluble compound, azoxystrobin, respectively. Overall, results suggest 2,4-D release from clippings varies across turfgrass species, with it being greatest from zoysiagrass and least from hybrid bermudagrass, while azoxystrobin release did not vary across species. Additional research is required to elucidate releasal mechanisms, and ultimately to determine if zoysiagrass provides comparably superior surface water protection potential from reduced 2,4-D release.
Conclusions

Currently, turfgrass pesticide labels currently registered for use on athletic fields, commercial properties, golf courses, and residential lawns provide very few, if any guidelines regarding clipping management following application. When guidelines are provided, they are typically focused on mowing periods before/after application to promote acceptable pest control, which is important; however, applicators should be informed of other effects from mowing in pesticide-treated areas. Data from this research suggest turfgrass clippings can serve as an off-target transport process for pesticides including 2,4-D and azoxystrobin up to 32 DAT. Additionally, these compounds can release from vegetation into water, which may deleteriously affect aquatic ecosystem health. This research further supports the currently recommended practice of returning clippings to the turfgrass stand when mowing, as removing 2,4-D and azoxystrobin from the system in clippings may reduce pest control and cause adverse off-target impacts. Under conditions that cause excessive clipping accumulation and require collection, turfgrass managers should implement practices to ensure clippings are not introduced into environmentally sensitive areas. Future research should investigate application timing and seasonal affects on retention and behavior of other pesticides in turfgrass clippings, as well as best management practices to reduce off-target transport via clipping collection. Additionally, research should quantify pesticide removal in turfgrass clippings on golf course putting greens, where routine clipping collection is required.
References


Yu, J., P.E. McCullough, and W.K. Vencill. 2013. Absorption, translocation, and metabolism of amicarbazone in annual bluegrass (Poa annua), creeping bentgrass (Agrostis stolonifera), and tall fescue (Festuca arundinacea). Weed Sci. 61:217-221.
Table 1. Physicochemical, physiological, and toxicological properties of 2,4-D and azoxystrobin.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Property</th>
<th>2,4-D Amine → Acid\textsuperscript{b}</th>
<th>Azoxystrobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticide class</td>
<td>Herbicide</td>
<td>Fungicide</td>
</tr>
<tr>
<td>Water solubility (mg L\textsuperscript{-1}; pH 7)</td>
<td>33,900 to 796,000</td>
<td>28 to 85</td>
</tr>
<tr>
<td>Hydrolysis half-life (d)</td>
<td>39</td>
<td>Stable</td>
</tr>
<tr>
<td>Aqueous photolysis half-life (d)</td>
<td>19</td>
<td>11 to 17</td>
</tr>
<tr>
<td>Plant uptake</td>
<td>Foliar/Root</td>
<td>Foliar/Root</td>
</tr>
<tr>
<td>Plant translocation</td>
<td>Acropetal</td>
<td>Acropetal</td>
</tr>
<tr>
<td>Aquatic invertebrates</td>
<td>Non to slight</td>
<td>Very high</td>
</tr>
<tr>
<td>Aquatic plants</td>
<td>Non</td>
<td>Moderate to high</td>
</tr>
<tr>
<td>Freshwater fish</td>
<td>Very high</td>
<td>Moderate to very high</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 2,4-D and azoxystrobin data obtained from EPA (2005) and EPA (1997), respectively.

\textsuperscript{b} Data presented for both due to rapid conversion of amine to acid in plants (EPA 2005).
Table 2. 2,4-D application timing-by-turfgrass species interaction on residue in collected turfgrass clippings.

<table>
<thead>
<tr>
<th>DBCC&lt;sup&gt;b,c&lt;/sup&gt;</th>
<th>Turfgrass species</th>
<th>% of applied</th>
<th>LSD&lt;sub&gt;0.05&lt;/sub&gt;</th>
<th>Turfgrass species</th>
<th>% of applied</th>
<th>LSD&lt;sub&gt;0.05&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>MST&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>MST&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>HB</td>
<td>378</td>
<td>429</td>
<td>NS</td>
<td>42.4</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>388</td>
<td>NS</td>
<td></td>
<td>32.9</td>
<td>19.3</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>247</td>
<td>303</td>
<td>7</td>
<td>27.8</td>
<td>16.9</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>182</td>
<td>265</td>
<td>26</td>
<td>20.4</td>
<td>16.9</td>
</tr>
<tr>
<td>4&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>45</td>
<td>69</td>
<td>NS</td>
<td>5.0</td>
<td>4.4</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>33</td>
<td>36</td>
<td>NS</td>
<td>3.7</td>
<td>2.3</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>25</td>
<td>18</td>
<td>2</td>
<td>2.8</td>
<td>1.1</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>21</td>
<td>7</td>
<td>3</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>24</td>
<td>17</td>
<td>8</td>
<td></td>
<td>2.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations: DBCC, d before clipping collection; HB, hybrid bermudagrass; TF, tall fescue; Z, zoysiagrass; NS, non-significant.

<sup>b</sup> Mowing did not occur from 4 to 0 DBCC 2,4-D application.

<sup>c</sup> Average fresh clipping harvest yield = hybrid bermudagrass (1.5 g m<sup>-2</sup>), tall fescue (1.1 g m<sup>-2</sup>) and zoysiagrass (0.8 g m<sup>-2</sup>).

<sup>d</sup> 7.5 mm H<sub>2</sub>O rainfall occurred 16 hours after 4 DBCC application.
Table 3. Azoxystrobin application timing-by-turfgrass species interaction on residue in collected clippings.\(^a\)

<table>
<thead>
<tr>
<th>DBCC(^b,c)</th>
<th>Turfgrass species</th>
<th>mg kg(^{-1})</th>
<th>LSD(_{0.05})</th>
<th>% of applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HB</td>
<td>TF</td>
<td>Z</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>81</td>
<td>71</td>
<td>81</td>
<td>NS</td>
</tr>
<tr>
<td>1</td>
<td>57</td>
<td>62</td>
<td>44</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>60</td>
<td>54</td>
<td>NS</td>
</tr>
<tr>
<td>4(^d)</td>
<td>16</td>
<td>34</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>18</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>1</td>
<td>&lt;LOD</td>
<td>NS</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: DBCC, d before clipping collection; HB, hybrid bermudagrass; TF, tall fescue; Z, zoysiagrass; NS, non-significant; LOD, limit of detection.

\(^b\) Mowing did not occur from 4 to 0 DBCC azoxystrobin application.

\(^c\) Average fresh clipping harvest yield = hybrid bermudagrass (1.5 g m\(^{-2}\)), tall fescue (1.1 g m\(^{-2}\)) and zoysiagrass (0.8 g m\(^{-2}\)).

\(^d\) 7.5 mm H\(_2\)O rainfall occurred 16 hours after 4 DBCC application.
Table 4. 2,4-D application timing before clipping collection interval-by-turfgrass species interaction and the main effect of azoxystrobin application timing on residue release into water 2 days after application of clippings collected from previously treated turfgrass.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Turfgrass</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DBCC</td>
<td>mg 2,4-D L\textsuperscript{-1}</td>
<td>% 2,4-D release\textsuperscript{b}</td>
<td>DBCC</td>
<td>mg Azoxy L\textsuperscript{-1}</td>
<td>% Azoxy release</td>
</tr>
<tr>
<td>Hybrid bermudagrass</td>
<td>10.0</td>
<td>2.2</td>
<td>1.5</td>
<td>35</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>19.1</td>
<td>3.6</td>
<td>2.6</td>
<td>41</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>Zoysiagrass</td>
<td>17.0</td>
<td>7.4</td>
<td>3.3</td>
<td>41</td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td>LSD\textsubscript{0.05}</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>All turfgrasses</td>
<td>0.91</td>
<td>0.50</td>
<td>0.29</td>
<td>10.1</td>
<td>15.2</td>
<td>14.8</td>
</tr>
<tr>
<td>LSD\textsubscript{0.05}</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: DBCC, days before clipping collection; Azoxy, azoxystrobin; NS, non-significant.
\textsuperscript{b} % release calculations based on pesticide residue in collected clippings.
CHAPTER 2: FACTORS INFLUENCING DISLODGEABLE 2,4-D PLANT RESIDUE FROM HYBRID BERMUDAGRASS (Cynodon Dactylon L. X C. Transvaalensis) ATHLETIC FIELDS.


Matthew D. Jeffries¹*, Travis W. Gannon¹, James T. Brosnan², Khalied A. Ahmed¹, and Gregory K. Breeden²

Research to date has confirmed 2,4-D residue may dislodge from turfgrass; however, experiments have not been conducted on hybrid bermudagrass (Cynodon dactylon L. x C. transvaalensis), the most common athletic field turfgrass in subtropical climates. More specifically, previous research has not investigated the effect of post-application irrigation on dislodgeable 2,4-D residue from hybrid bermudagrass and across turfgrass species, research has been nondescript regarding sample time within a d (TWD) or conducted in the afternoon when the turfgrass canopy is dry, possibly underestimating potential for dislodgement. The effect of irrigation and TWD on 2,4-D dislodgeability was investigated. Dislodgeable 2,4-D amine was reduced > 300% following irrigation. From 2 to 7 d after treatment (DAT), ≤ 0.5% of applied 2,4-D was dislodged from irrigated turfgrass, while ≤ 2.3% of applied 2,4-D was dislodged when not irrigated. 2,4-D dislodgeability decreased as TWD increased. Dislodgeable 2,4-D residue declined to < 0.1% of the applied at 1 DAT – 13:00, and increased to 1 to 3% of the applied 2 DAT – 5:00, suggesting 2,4-D re-suspended on treated turfgrass vegetation overnight. In conclusion, irrigating treated turfgrass reduced
dislodgeable 2,4-D. 2,4-D dislodgeability increased as TWD decreased, which was attributed to non-precipitation climatic conditions favoring turfgrass canopy wetness. This research will improve turfgrass management practices and research designed to minimize human 2,4-D exposure.

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Introduction

Turfgrasses are grown on over 16.3 million hectares in the contiguous United States (US) – exceeding the combined area of irrigated grain corn [(Zea mays L.) 2.5 million], soybeans [Glycine max L.] 2.1 million] and cotton [(Gossypium hirsutum L.) 0.9 million] – and are utilized by the public with land uses including commercial/residential lawns, golf courses, parks and roadsides [1, 2]. Turfgrasses on athletic grounds and facilities are also widespread, with > 700,000 managed athletic fields in 2003 [3]. The US Census Bureau reported in 2009 over 40% of the population ages 7 to 44 participated in baseball, football, golf, soccer and/or softball, all played predominately on managed natural turfgrass [4]. Providing an acceptable playing surface poses many challenges for athletic field managers. The surface must be aesthetically pleasing, functional and safe to the end-user. Public athletic fields are often overused or used when environmental conditions favor playing surface degradation. Consequently, weed encroachment may occur that adversely affects surface strength and uniformity. Ultimately, player safety may be compromised due to poor footing conditions manifesting as playing surfaces degrade [5, 6]. To mitigate these issues, synthetic herbicides are commonly applied for weed control on athletic fields.

Major pathways of direct human exposure to pesticides in turfgrass systems include inhalation (dust/vapor), nondietary ingestion and dermal contact [7]. 2,4-dimethylamine salt (2,4-D) is a selective postemergence broadleaf herbicide currently registered for use in numerous US crops and non-cropland areas. In 2005, over 650 products containing 2,4-D were registered in over 300 distinct agricultural and residential use sites [8]. At that time, it was reported 7.3 million kg 2,4-D (34% of total US use) were applied to non-cropland areas
including athletic fields [8]. While 2,4-D is routinely applied in numerous commodities worldwide, its use has been questioned since the 1970’s due to toxicological concerns [9, 10]. Research regarding 2,4-D carcinogenicity, as well as its effect on neurologic and reproductive processes is inconclusive; however, it is a known toxin to blood (reduction in hemoglobin and red blood cells), the liver (decreased enzyme activity) and the kidney (increased organ weight) [8, 11, 12]. Furthermore, acute 2,4-D exposure is an eye irritant and chronic 2,4-D oral exposure experiments on animals have resulted in damage to the eye, thyroid, kidney, adrenals and ovaries/testes [8].

Following a pesticide application to turfgrass, numerous transfer and transport processes ensue. Chemical properties pertaining to 2,4-D transport from the intended site include: very high water solubility ($K_w = 796,000 \text{ mg L}^{-1} \text{ L} \cdot \text{mg}^{-1}$; $20^\circ \text{ C}$), short soil half-life ($T_{1/2} = 6.2 \text{ d}$), low volatility (vapor pressure = $1.0 \times 10^{-7} \text{ mm Hg}$) and moderate soil organic carbon sorption coefficient ($K_{oc} = 20 \text{ mL g}^{-1}$) [8,13,14]. These properties suggest 2,4-D may readily dislodge from treated turfgrass vegetation onto humans, which has been confirmed most notably by Nishioka et al. [15] and Morgan et al. [16]. In these independent studies, samples were collected from various areas within homes prior to, and following 2,4-D applications to the residential lawns. The authors detected 2,4-D in 122 of the 142 homes sampled (across two states and three populations) [15, 16]. These data confirm 2,4-D transfers from turfgrass vegetation to off-target areas since it was not applied inside, nor is labeled for indoor use. If transferred from treated turfgrass vegetation, 2,4-D human non-occupational absorption occurs via dietary and non-dietary ingestion, and to lesser extent through skin [15–18]. Once 2,4-D is absorbed into the body, it is not metabolized and is rapidly eliminated in urine [16].
For this reason, human 2,4-D exposure is typically measured via urine samples, and has been commonly detected in children’s urine, confirming exposure [16, 19, 20].

Unlike most traditional agricultural settings, non-occupational re-entry into areas recently treated with pesticides is common and lawful in most turfgrass systems throughout many regions of the US. In most states, a specific non-occupational re-entry interval is not required following a pesticide application to athletic field turfgrass. With many pesticides, as long as the product has dried, re-entry is permissible. Plant canopies in established turfgrass systems inherently intercept sprayed pesticides. Pesticide in/on turfgrass vegetation is subject to dislodge onto maintenance equipment or players, increasing human pesticide exposure potential [21]. Typically, due to reported short half-lives (< 7 d) in turfgrass vegetation, pesticide dislodgement off of the treated area is not considered a long-term concern; however, appreciable amounts can be dislodged in the h and d immediately following an application [22, 23]. Sears et al. [24] reported 10% of the applied diazinon was dislodged by wiping cheesecloth over the treated area immediately after application with ten strokes in opposing directions. Similarly, Harris and Solomon [25] conducted an experiment simulating a recreational setting and reported 8% of the applied 2,4-D was dislodged onto running shoes 1 h following application.

Previous research indicates dislodgeable pesticide residue on plants may be reduced by applying granular products in lieu of spray applications, as well as by irrigating treated areas following an application [21, 26, 27]. Thompson et al. [28] reported liquid applied 2,4-D was up to 15 times more dislodgeable than granular 2,4-D following an application. The authors also reported < 0.01% of the applied 2,4-D liquid formulation was dislodgeable after
1 d when a rainfall event (18 mm) occurred 1 h after treatment, suggesting post-application irrigation may reduce dislodgeable 2,4-D plant residue [28]. While this information is valuable for reducing dislodgeable 2,4-D plant residue, it should be noted granular applied 2,4-D and irrigation/rainfall 1 h after spraying 2,4-D may compromise weed control [29, 30]. Additional research is needed to evaluate dislodgeable 2,4-D plant residue when applied as a liquid formulation and irrigation is delivered at an agronomically sound timing.

To our knowledge, research to date has not evaluated dislodgeable 2,4-D plant residue on hybrid bermudagrass athletic fields. Hybrid bermudagrass is the most commonly managed turfgrass species on athletic fields in tropical and subtropical (latitudes ≈ 45° N to 45° S) regions of the world due to its tolerance to low mowing heights (≥ 0.6 cm) coupled with its comparatively superior recuperative abilities following periods of heavy traffic [31]. Currently, published 2,4-D dislodgement research has predominantly been conducted on Kentucky bluegrass (*Poa pratensis* L.), a cool-season turfgrass species that possesses different growth characteristics than bermudagrass [31]. Specifically, Kentucky bluegrass has an erect, bunch-type growth habit and a C₃ photosynthetic pathway. The presented research was conducted on a prostrate growing (rhizomes/stolons), C₄ turfgrass. In addition, previous research has shown turfgrass species with similar growth characteristics commonly differ with regards to herbicide uptake, translocation and metabolism, which further supports the need for turfgrass species-specific research on dislodgeable pesticide residue [32–34].

Research has not been published quantifying pesticide dislodgeability from turfgrass over time within a d (TWD). In many regions, turfgrass canopy dynamics, including surface moisture, fluctuate throughout the day. As moisture increases, compounds may re-suspend
on turfgrass vegetation, and therefore may be more dislodgeable if not tightly bound to vegetation (i.e. high water solubility and low to moderate binding affinity). Finally, published research to date has not encompassed 2,4-D dislodgeability in a setting simulating the most popular international sport, soccer [35]. Beyond its popularity, research evaluating 2,4-D dislodgeability is warranted due to the inherent frequency of ball-to-turfgrass contact that occurs in this sport. If 2,4-D dislodges from treated turfgrass onto the ball human exposure may occur via numerous routes, most notably by hand contact during certain procedures within games/practices.

The objectives of this research were to quantify dislodgeable 2,4-D plant residue over time on a hybrid bermudagrass athletic field surface, as well as to elucidate the effect of irrigation and natural surface moisture on 2,4-D dislodgeability from hybrid bermudagrass turfgrass. We hypothesized irrigation and conditions favoring turfgrass canopy dryness would reduce 2,4-D dislodgeability on athletic fields.

**Materials and Methods**

**Research Overview**

Two field experiments were conducted and repeated in time in North Carolina to evaluate dislodgeable 2,4-D plant residue from hybrid bermudagrass. Experiment 1 was designed to quantify the effect of irrigation on dislodgeable 2,4-D plant residue. 2,4-D-treated plots were either irrigated (0.3 cm H2O) or not irrigated 24 h following treatment. Samples characterizing 2,4-D dislodgement from turfgrass were collected from 7:00 to 9:00 EST at various d after treatment (DAT). Results from this experiment suggested
dislodgeable 2,4-D residue declined over time within a sampling date, which we hypothesized was due to a reduction in turfgrass canopy moisture as dew/distillation/guttation fluid dissipated off vegetation. To investigate this observation, an additional experiment was developed. Experiment 2 was designed to quantify the effect of TWD on dislodgeable 2,4-D plant residue. In short, 2,4-D treated plots were sampled every 2 h from 5:00 to 13:00 EST at various DAT to elucidate the effect of turfgrass canopy surface moisture on dislodgeable 2,4-D residue.

**Site Description**

Experiment 1 was initiated June 10, 2013 and May 28, 2014 (Thomas E. Brooks Park, Cary, NC; Lat. 35°47’41.74” N, Long. 78°53’50.37” W) on a sand textured soil with pH 6.9 and 1.5% organic matter (OM) w w⁻¹. Experiment 2 was initiated August 27, 2013 and August 26, 2014 (Lake Wheeler Turfgrass Field Lab, Raleigh, NC; Lat. 35°44’21.34” N, Long. 78°40’49.75” W) on a sandy clay loam soil with pH 6.4 and 1.9% OM w w⁻¹.

Both experiments were conducted on weed-free, established hybrid bermudagrass [Cynodon dactylon (L.) Pers. × Cynodon transvaalensis Burtt-Davy, cv. ‘Tifway 419’] areas maintained at a 3 cm height of cut where 2,4-D had not been applied 2 yr preceding initiation. Prior to experiment initiation, vegetation and soil samples from the areas were analyzed to confirm 2,4-D residue were non-detectable. Select climatic conditions were logged throughout experiments. Finally, leaf wetness (Leaf Wetness Sensor; Decagon Devices Inc., Pullman, WA) was measured throughout experiment 2 with a flat-plate sensor placed facing north at a 45° angle from the ground surface and 0.6 m height.
**Experimental Design**

Experiment 1 was conducted as a split plot, randomized complete block design with three replicates of a 2-by-7 factorial treatment arrangement. Main plots were split by irrigation (irrigated or non-irrigated) with seven subplot sample timings (1, 2, 5, 7, 14, 21 or 28 DAT). Experiment 2 was conducted as a randomized complete block design with three replicates of a 5-by-5 factorial treatment arrangement. Factors included sample collections at five TWD (5:00, 7:00, 9:00, 11:00 or 13:00 EST) in each of five DAT (1, 2, 3, 6 or 12 DAT). Samples were also collected for both experiments 1 h after application and immediately following application for experiment 2; however, these samples were not included in statistical analyses due to differing collection timings from other samples beyond 0 DAT. A nontreated check was included in all experimental blocks to ensure the trial area was not contaminated.

**Experiment Initiation**

One d prior to trial initiation, areas were mown (clippings collected) and irrigated to field capacity. Experimental areas were not irrigated or mown for 7 d following treatment. Furthermore, areas were covered (HDX 6 Mil Clear Plastic; The Home Depot Corp., Atlanta, GA) during rainfall events during this 7 d period. At experiment initiation, 2,4-D amine (Amine 400 2,4-D Weed Killer®; PBI/Gordon Corp., Kansas City, MO) was applied at 2.1 kg ai ha\(^{-1}\) to plots measuring 1.5 by 2.25 m (1 m alleys between reps). Treatments were sprayed at 14:00 to allow the solution to dry on the vegetation during \(\geq 5\) h of sunlight. Applications were made with a hand-held CO\(_2\)-pressurized sprayer comprised of four 80015 XR VS flat-fan nozzles (TeeJet® Flat-Fan Nozzles; Sprayi
volume selected (187 L ha\textsuperscript{-1} at 179 kPa) is the minimum stated on the label, creating the worst-case scenario for pesticide retention on the turfgrass canopy. Also, it should be noted that the 2,4-D application rate used in this research was 20% higher than currently allowed on athletic fields. This was done based on preliminary testing that confirmed an increased rate was necessary to ensure 2,4-D residue detection up to 7 DAT, which we felt was a justifiable compromise to better elucidate the effects of the research variables of interest.

Finally, to ensure 2,4-D was applied at the intended rate over the trial area, water-filled glass containers (15.5 cm\textsuperscript{2}; 65 mL HPLC-grade H\textsubscript{2}O) were randomly placed throughout the trial area. Following 2,4-D application overtop containers, the residue in these containers was quantified by high performance liquid chromatography (HPLC) with a diode array detector (DAD) analysis.

Irrigation treatments for experiment 1 were applied at 1 DAT (13:00 EST) with an eight-nozzle boom equipped with 8008 XR VS flat-fan nozzles that was calibrated at 172 kPa to deliver 0.3 cm H\textsubscript{2}O plot\textsuperscript{-1} with four passes. This approach to simulate irrigation was conducted due to logistical considerations for the research area and experimental design, as well as to ensure uniform irrigation amount/intensity and minimize variability in the data. Furthermore, the output rate and the droplet size produced by the nozzle-pressure combination of our boom both fall within the spectrum delivered by various commercially available impact and rotary style irrigation heads in turfgrass systems [36–38].
Sample Collection

Total 2,4-D in/on Turfgrass Vegetation

Total 2,4-D in/on turfgrass vegetation was quantified at all sample collection timings to: 1) use as a reference point for the total amount of 2,4-D dislodged from hybrid bermudagrass over time (section 2.7, equation 1); and 2) characterize 2,4-D dissipation in/on hybrid bermudagrass over time. This was done by collecting a core (10.8 cm diam; 92 cm²) such that sampling equipment did not contact aboveground vegetation. Following collection, all samples were frozen, aboveground vegetation was harvested, weighed, processed [1.7 mm (Fitzmill Homoloid Model JT 6; Fitzpatrick Co., Elmhurst, IL)] and stored at -12°C until extraction and residue analysis.

Total Dislodgeable 2,4-D

Dislodgeable 2,4-D was quantified by rolling a soccer ball (Franklin Sports Competition 100 Soccer Ball, Size 4; Franklin Sports, Stoughton, MA) over a 9 m distance (four 2.25 m side-by-side rolls) within a unique plot. Ball roll distance was based off of a soccer ball rolling 50% of the recommended field length (18 m) for youth ages < 6 yr in the US [39]. The soccer ball was double-wrapped with a 5 by 120 cm absorbent strip (100% Cotton Cheesecloth; Chef Revival, North Charleston, SC) selected by previous researchers investigating related processes [24, 25, 28]. The soccer ball was mounted to a hand-held PVC apparatus designed such that the ball rotated end over end in the same direction as the absorbent strip, thus allowing for constant absorbent strip contact to the treated turfgrass surface. While this is not representative of a ball roll when actively playing soccer, this measure was required to minimize variation in data and determine the maximum
dislodgeable 2,4-D from turfgrass vegetation. Following ball roll, the absorbent strip was removed, placed in a unique glass jar (473 cm$^3$) and stored at -12° C for subsequent extraction and HPLC-DAD analysis.

Residue Analyses

Total 2,4-D in/on Turfgrass Vegetation

All reagents and solvents used for residue analyses were HPLC grade. Total 2,4-D residue in/on turfgrass vegetation was quantified with modifications to methods by Shin et al. [40]. Processed vegetation (5 g) was mixed with water (100-120 mL) in a high-speed homogenizer [9000 rpm (VIRTIS 45 Homogenizer; The VIRTIS Co., Gardiner, NY)] for 2 min. After mixing, samples settled (10 min) and an aliquot (10 mL) was centrifuged [3500 rpm (Allegra 6-KR Centrifuge; Beckman Coulter Inc., Brea, CA)] for 10 min. A sub-aliquot (7 mL) was then taken and sodium chloride (1 g) was added and partitioned with n-hexane (4 mL). The upper hexane layer was discarded and this partition was repeated once. The aqueous solution was acidified with 10% sulfuric acid (0.5 mL) to reach pH < 2. The acidified aqueous solution was partitioned twice with dichloromethane [6 mL total (DCM)] and evaporated to dryness using N-evap (N-EVAP 112; Organomation Associates Inc., Berlin, MA). Samples were reconstituted in water + acetonitrile [7 mL (9 + 1 by volume)]. Samples were sonicated for 3 min (Branson 2510 Ultrasonic Cleaner; Branson Ultrasonic Co., Danbury, CT) and vortexed for 3 min. Samples were then vialed for injection.

Total Dislodgeable 2,4-D

Total dislodgeable 2,4-D residue was quantified with modifications to methods by Snyder and Cisar. [15] Prior to extraction, samples were allowed to thaw at room temperature
(22° C) for 30 min. Water (100 mL) was added to each sample and shaken [300 rpm (KS 501 Digital Shaker; IKA Works, Inc., Wilmington, NC)] for 1 h. After shaking, extract was decanted, the absorbent strip was compressed to remove additional solution and the extraction process was repeated with water (25 mL). Extracts were combined and mixed for 5 min and an aliquot (50 mL) was taken and centrifuged (3500 rpm) for 10 min. Each centrifuged sample (1 mL) was filtered (0.45 µm nylon filter; Thermo Fisher Scientific, Inc., Pittsburgh, PA) and vialled for injection.

**Analytical Parameters**

2,4-D residue was quantified for cheesecloth, vegetation and water matrices by HPLC-DAD (Agilent-1260 Infinity; Agilent Technologies, Inc., Wilmington, DE). High performance liquid chromatography parameters included: C<sub>18</sub> silica column [75 mm length by 4.6 mm i.d. (Poroshell 120 EC-C18; Agilent Technologies, Inc., Wilmington, DE)]; 32° C column temperature; acetonitrile + water (3 + 2 by volume) + 0.1% phosphoric acid by volume mobile phase; 1 mL min<sup>−1</sup> flow rate; 10 µL injection volume. 2,4-D retention time was 0.6 min at 230 nm. Limits of detection and quantification were 0.3 and 1.0 mg L<sup>−1</sup> respectively, while maintaining the signal to noise ratio at 3:1. Pesticide residue was quantified using peak area measurements (OpenLAB CDS ChemStation, Version C.01.04; Agilent Technologies, Inc., Wilmington, DE). Concentrations above the calibration curve were diluted and re-injected for analysis. Finally, fortification recovery checks for cheesecloth, vegetation and water matrices ranged from 90 to 101, 85 to 97 and 98 to 102%, respectively, across all analyses conducted in the presented research.
Dislodgement Calculations

From the data collected, 2,4-D dislodgeability is presented in two ways. The first is only reported for experiment 1, which is dislodgement relative to the total amount in/on turfgrass vegetation at a given point in time that was calculated using the equation:

\[
\text{dislodgment} = \left( \frac{\text{BR } 2,4\text{-D cm}^{-2}}{\text{AV } 2,4\text{-D cm}^{-2}} \right) \times 100
\]

where BR and AV represent 2,4-D residue recovered from ball roll and turfgrass vegetation samples, respectively. The second reporting method was completed for ball roll and turfgrass vegetation recoveries in both experiments, which was 2,4-D dislodgement relative to the amount applied at trial initiation using the equation:

\[
\text{dislodgment} = \left( \frac{\text{BR } 2,4\text{-D cm}^{-2}}{20.9 \mu g \text{ 2,4-D cm}^{-2}} \right) \times 100
\]

where BR represents 2,4-D residue recovered from ball roll samples relative to the 2,4-D application rate (20.9 \( \mu g \ 2,4\text{-D cm}^{-2} \)).

Statistical Analysis

Statistical analyses were conducted by analysis of variance (P = 0.05) using MIXED procedures in SAS (Statistical Analysis Software®, Version 9.2; SAS Institute, Inc., Cary, NC). Irrigation and TWD were considered fixed effects for experiments 1 and 2, respectively, while DAT was considered a fixed effect for both experiments. Main effects
and their interactions are presented accordingly, with precedent given to significant
interactions of increasing magnitude [42]. Means were separated according to Fisher’s
protected LSD (P < 0.05) and Pearson correlation coefficients (P = 0.05) were determined to
quantify the relationships between selected climatic conditions and leaf wetness with
dislodgeable 2,4-D plant residue.

Results

Application Recovery Checks and Experimental Runs

Application recovery check containers determined 2,4-D was applied at 93 to 106%
of the intended rate for all experiments and experimental runs. In general, data trends
between runs of experiment 1 were similar; however, dislodgeable 2,4-D residue was
detected for a longer period of time following application in run 2. The authors attribute this
to improved methods for covering plots during periods of rainfall in run 2. Plots were
covered in run 1 of both experiments by securing plastic to the ground during rainfall events.
While turfgrass vegetation was always dry when plots were covered, ground distillation
coupled with evapotranspiration caused the accumulation of water droplets on the underside
of the plastic. Although measures were taken to minimize this occurrence and prevent
contamination across plots, there was likely an effect on dislodgeable 2,4-D residue via
losses sorbed to plastic or soil/thatch. To address this concern, PVC structures were
constructed prior to run 2 of both experiments to prevent plastic contact with treated turfgrass
vegetation during rainfall events. Consequently, experimental runs were analyzed and are
presented separately for experiment 1, while experimental runs were pooled for experiment 2.

**Effect of Irrigation on 2,4-D Dislodgeability**

*Dislodge of Total in/on Turfgrass Vegetation*

2,4-D residue was not detected on turfgrass vegetation beyond 7 DAT; therefore, data from 14 to 28 DAT were excluded from statistical analyses. Overall, 2,4-D dislodgement as a percent of the total in/on turfgrass vegetation at a given point in time was negligibly affected by irrigation or DAT (Table 1). In run 1 an irrigation-by-DAT interaction was detected; however, no differences were detected between irrigation treatments at 2 DAT, which is the first sampling following irrigation. However, in run 2 a greater proportion of the total 2,4-D in/on turfgrass vegetation was dislodged from non-irrigated plots at 2 and 5 DAT. Across runs, no differences were detected at 7 DAT.

While minimal differences in dislodgment were detected as a percentage of the total load of 2,4-D in/on turfgrass vegetation, the quantity of 2,4-D in/on turfgrass vegetation was affected by irrigation and DAT in both runs (Table 2). In neither experimental run were differences detected prior to irrigation (i.e., 1 DAT sampling); however, non-irrigated plots retained more 2,4-D at 2 and 5 DAT compared to irrigated vegetation. In run 1, 2,4-D retention was reduced 72% at 2 DAT by irrigating plots. Furthermore, < 7% of applied 2,4-D remained in/on turfgrass vegetation at 5 and 7 DAT when irrigation was applied. Although less pronounced in run 2, all irrigated samples collected from 2 to 7 DAT retained less 2,4-D than non-irrigated vegetation. Finally, 2,4-D was not detected in/on turfgrass vegetation from 14 DAT until the end of the study in both experimental runs.
**Dislodge of Applied**

In both experimental runs, dislodgeable 2,4-D residue (relative to the applied) was reduced > 58% following irrigation (Table 3). At 2 DAT and beyond, ≤ 0.5% of applied 2,4-D was dislodged from irrigated vegetation. On non-irrigated plots, > 1.5% of applied 2,4-D was dislodged 2 DAT (in both experimental runs), as well as 5 and 7 DAT in run 2. Finally, data from this research suggested 2,4-D dislodgeability fluctuated after application. At 0 DAT – 13:00 (1 h following treatment when vegetation had dried) 0.3% of the applied was dislodged (data not shown), which increased to 2.1% the following morning. From this observation the effect of TWD was further elucidated.

**Effect of Time Within a Day on 2,4-D Dislodgeability**

**Dislodge of Applied**

2,4-D residue was not detected on turfgrass vegetation beyond 6 DAT; therefore, data from 12 DAT were excluded from statistical analyses. 2,4-D residue was not detected beyond 3 and 6 DAT in run 1 and 2 respectively; therefore, data beyond these times were excluded from statistical analyses. A significant DAT-by-TWD interaction was detected (Table 4). In general, 2,4-D dislodgeability decreased as TWD increased (within a DAT), and decreased as DAT increased (within a TWD). Maximum dislodgement was observed at 1 DAT – 5:00, 7:00 and 9:00, with 3.6 to 4.0% of applied 2,4-D dislodged. At these three TWD timings, 2,4-D dislodgement decreased from 1 to 6 DAT; however, no differences were detected between timings within a DAT. With the exception of two sample timings (1 and 3 DAT – 11:00), ≤ 0.1% of applied 2,4-D was dislodged at 11:00 and 13:00 from 1 to 6 DAT. When comparing TWD across DAT in both experimental runs, data suggested 2,4-D
re-suspended on turfgrass vegetation overnight, as 2,4-D dislodgement at 1 DAT – 13:00 (0.1% of applied 2,4-D) was less than 2 DAT – 5:00 (2.1%; Fig 1). While not statistically significant, there was also a ten-fold increase in 2,4-D dislodged from 2 DAT – 13:00 (0.1% of applied 2,4-D) to 3 DAT – 5:00 (1%).

_Climatic Condition Correlations with Dislodgeability_

A review of climatic conditions from 0 to 6 DAT suggested 2,4-D dislodgement may be influenced by climatic conditions that affect turfgrass canopy moisture (Table 5). Relative humidity (RH) is a dimensionless ratio, expressed as a percent of the amount of atmospheric moisture present relative to saturated air [43]. Dew point (DP) is the air temperature (AT) below which moisture in the air condenses, forming dew [44]. Baier [45] and Wilson et al. [46] reported peak dew formation occurred at, or just beyond, sunrise. Hughes and Brimblecombe [45] reported dew formation on velvetgrass (*Holcus lanatus* L.) over a 7 mo period was never observed without guttation, or the emergence of liquid within plants via hydathodes along leaf margins. While these parameters do not solely influence turfgrass canopy moisture, increasing RH, decreasing differences between AT and DP and decreasing time from sunrise (TFS) suggest conditions become more favorable for turfgrass canopy moisture development. Following canopy moisture development, 2,4-D may more readily dislodge from vegetation into solution on treated turfgrass surfaces, and residue may be more readily transferred onto soccer balls. From 1 to 3 DAT, RH was positively correlated with dislodgeable 2,4-D residue (*r* = 0.57 to 0.69; *P* ≤ 0.01), while negative correlations were observed between dislodgeable 2,4-D and the difference between AT and DP (*r* = -0.55 to -0.73; *P* ≤ 0.01) as well as TFS (*r* = -0.58 to -0.82; *P* ≤ 0.001) (Table 6). Correlations
weakened as DAT increased, with decreases in all aforementioned comparisons to < 0.5 (+/-) at 6 DAT. Finally, 2,4-D dislodgeability was poorly to moderately positively correlated with leaf wetness from 1 to 6 DAT ($r = 0.23$ to 0.58).

**Discussion**

Data from this research align with previous reports that irrigation following foliar pesticide applications to turfgrass significantly reduce dislodgeable residue [24, 26, 27, 48]. Thompson et al. [28] reported dislodgeable 2,4-D declined to < 0.01% of the applied after a rainfall event 1 h after treatment. The less pronounced effect of water inputs in the presented research is likely due to a longer period of time between 2,4-D application and irrigation/rainfall (23 h). Although it cannot be determined from our data how 2,4-D was proportionally reduced in or on turfgrass vegetation following irrigation, this management practice significantly reduced the total 2,4-D in/on turfgrass vegetation at all sample timings. Averaged over experimental runs, irrigation reduced the total load of 2,4-D in/on turfgrass vegetation by 56% 2 DAT. This agrees with a previous report that 50% of applied dicamba, a synthetic auxin herbicide with physicochemical properties similar to 2,4-D, was lost via washoff after an 8 mm rainfall event [49].

Across irrigation treatments and sample collection timings, few differences were detected when reporting 2,4-D dislodgement as a percent of the total load in/on turfgrass vegetation. This can be misleading from a human exposure perspective because of the reference point that dislodgeable 2,4-D was calculated from, as there were differences between the total 2,4-D in/on turfgrass vegetation. This suggests that over time there is a
general proportion of the total 2,4-D load in/on turfgrass vegetation that was dislodgeable via soccer ball roll and ultimately, management practices should be implemented to reduce these loads to limit human exposure.

Data from the TWD experiment suggested 2,4-D dislodgeability declined from morning to afternoon, which is hypothesized to be in part from turfgrass canopy moisture development. Poor to moderate correlations between dislodgeability and leaf wetness is attributed in part to sub-optimization of sensor placement (0.6 m height) in relation to the turfgrass canopy (0.03 m height). Kruit et al. [50] reported grass leaf wetness detection accuracy improved nearly 2-fold when sensors were moved from 1 to 0.1 m. Furthermore, previous research has shown turfgrass leaf wetness is commonly underestimated with data collected via flat-plate leaf wetness sensors [51, 52]. When using the RH threshold (71%) for grass moisture development determined by Kruit et al. [50], RH recordings in the presented research support 2,4-D dislodgeability is affected by canopy moisture. While this threshold will likely vary based on site-specific conditions, the strong positive correlations between RH and 2,4-D dislodgeability at 1 and 2 DAT in the presented research support this climatic parameter is an influencing factor. Additionally, 2,4-D dislodgement increased as AT approached DP and TFS decreased. Although it was not directly measured in this research, the aforementioned climatic parameters are predominately associated with dew formation on the turfgrass canopy. Once dew forms on turfgrass, 2,4-D may re-suspend on turfgrass vegetation, making it more readily dislodged. In addition to re-suspension, previous research has detected pesticides in plant guttation [53, 54]. Due to 2,4-D’s
acropetal movement in plants, guttation may contribute to increased dislodgement detected at 5:00, 7:00 and 9:00 [14].

Previously published pesticide dislodgment research in turfgrass has not emphasized the TWD that sample collection occurs. Reports either do not state specifically when samples were collected, or they were collected at times later than 10:50 [21, 24, 26–28, 55]. Furthermore, risk assessments for human pesticide exposure from treated turfgrass make calculations based on the total h per d an individual is exposed to a treated area [56, 57]. For example, the only study specifically evaluating human 2,4-D exposure from treated turfgrass in the 2005 US Environmental Protection Agency’s (EPA) re-registration of 2,4-D was conducted at 12:30:00 (1 and 24 h after application) [8, 25]. Data from our research suggest 2,4-D risk assessments may be improved if more specificity is provided when calculating exposure potential by including atmospheric and turfgrass canopy conditions.

To better communicate potential human 2,4-D exposure from this research, 2,4-D exposure d\(^{-1}\) was calculated with the algorithm obtained from the 2012 EPA Standard Operating Procedures for Residential Pesticide Exposure (Post-Application dermal Exposure – Physical Activities on Turf):

\[ E = \text{TTR}_t \times \text{CF1} \times \text{TC} \times \text{ET} \]

where \( E \) = exposure (mg d\(^{-1}\)); \( \text{TTR}_t \) = turf transferable residue on day \( t \) (0.82 \( \mu \)g cm\(^2\)); \( \text{CF1} \) = unit conversion factor (0.001 mg \( \mu \)g\(^{-1}\)); \( \text{TC} \) = transfer coefficient (49,000 cm\(^2\) hr\(^{-1}\); 1 to 2 yr children); and \( \text{ET} \) = exposure time (1.5 hr d\(^{-1}\); 1 to 2 yr children) in the algorithm [57]. The algorithm, \( \text{TC} \) and \( \text{ET} \) were obtained from the EPA risk assessment, while \( \text{TTR}_t \) (0.82 \( \mu \)g 2,4-D cm\(^2\)) corresponds to the maximum amount dislodged in the presented research (1 DAT
– 5:00) [57]. It was calculated that a human would be exposed to 60 mg 2,4-D d\(^{-1}\), which is adjusted to 6 mg after a 10% 2,4-D dermal absorption rate [8, 57]. Using the EPA risk assessment value for short-term (30 d) human dermal exposure of 25 mg kg\(^{-1}\) d\(^{-1}\) it, was determined that an average 1 to 2 yr child (11 kg) may be dermally exposed to 275 mg 2,4-D d\(^{-1}\) without adverse effect [8]. By calculating our observed maximum daily exposure (6 mg 2,4-D d\(^{-1}\)) as a percent of the short-term daily dermal exposure allowance without adverse effect (275 mg 2,4-D d\(^{-1}\)), it was determined that 2,4-D dislodged from one ball roll over a 0.45 m\(^2\) area equaled 2.2% of this limit. While the application rate in the presented research was 20% greater than current label allowances on athletic fields, which overestimates real-world exposure with this algorithm, the area covered by one ball roll in this research equals 0.18% of the area of the smallest children’s (U6) soccer field (14 by 18 m) recommended by the US Youth Soccer Organization [39].

**Conclusions**

This research evaluated 2,4-D dislodgement from hybrid bermudagrass, the most common athletic field turfgrass in tropical and subtropical regions, with a method simulating a common process in soccer, the most popular international sport. Our findings indicate that 2,4-D residue can dislodge from hybrid bermudagrass up to 7 DAT. Ultimately, 2,4-D’s very high \(K_s\) coupled with low \(K_{oc}\) have a substantial impact on dislodgeability, as irrigation reduced dislodgement > 68% and conditions favoring turfgrass canopy dryness reduced dislodgement to \(< 0.1\%\) of the applied from 2 DAT until the end of the study, regardless of irrigation.
In conclusion, human 2,4-D exposure on hybrid bermudagrass athletic fields may be minimized by the coordination of pesticide applications with event scheduling. By coupling the effect of irrigation after an application with dislodgeability decreasing when the turfgrass canopy is dry, data suggest 2,4-D may not be dislodgeable in the afternoon at, or beyond 2 DAT. However, without further research to investigate this observation, workers and non-workers should enter bermudagrass athletic fields recently treated with 2,4-D with caution in the days following an application. Future research should investigate similar research objectives with irrigation applied in the morning when 2,4-D is most dislodgeable, evaluate dislodgeability of additional pesticides from alternative turfgrass species, as well as the effect of adjusting application practices such as spray nozzle or carrier volume to reduce dislodgeable pesticide residue from turfgrass.
References


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Table 1. The effect of irrigation on dislodgeable 2,4-D relative to the total in/on aboveground vegetation.\(^a\)^\(^c\)

<table>
<thead>
<tr>
<th>DAT</th>
<th>Irrigated</th>
<th>Non-irrigated</th>
<th>Irrigated</th>
<th>Non-irrigated</th>
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\(^a\) Abbreviations: DAT, days after 2,4-D treatment; ND, non-detection.

\(^b\) 0.3 cm H\(_2\)O irrigation applied at 1 DAT (13:00 EST) after sampling.

\(^c\) Data from 14 to 28 DAT not included in statistical analysis.

\(^d\) LSD (P < 0.05) for comparing irrigation and DAT within run.
Table 2. The effect of irrigation on 2,4-D in/on aboveground vegetation.

<table>
<thead>
<tr>
<th>DAT</th>
<th>Run 1 Irrigated</th>
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</table>

\[ \text{LSD}_{0.05} = \begin{array}{c} 14 \\ 8 \end{array} \]

\( a \) Abbreviations: DAT, days after 2,4-D treatment; ND, non-detection.

\( b \) 0.3 cm H\(_2\)O irrigation applied at 1 DAT (13:00 EST) after sampling.

\( c \) Data from 14 to 28 DAT not included in statistical analysis.

\( d \) LSD (P < 0.05) for comparing irrigation and DAT within run.
Table 3. The effect of irrigation on dislodgeable 2,4-D relative to the applied at trial initiation.\textsuperscript{a-c}

<table>
<thead>
<tr>
<th>DAT</th>
<th>Irrigated</th>
<th>Non-irrigated</th>
<th>Irrigated</th>
<th>Non-irrigated</th>
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</table>

\textsuperscript{a} Abbreviations: DAT, days after 2,4-D treatment; ND, non-detection.

\textsuperscript{b} 0.3 cm H\textsubscript{2}O irrigation applied at 1 DAT (13:00 EST) after sampling.

\textsuperscript{c} Data from 14 to 28 DAT not included in statistical analysis.

\textsuperscript{d} LSD (P < 0.05) for comparing irrigation and DAT within run.
Table 4. The effect of time within a day on dislodgeable 2,4-D relative to the applied at trial initiation.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Time within a day\textsuperscript{c}</th>
<th>Days after treatment</th>
<th>% of applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>LSD\textsubscript{0.05}\textsuperscript{d}</td>
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\textsuperscript{a} Abbreviation: ND, non-detection.
\textsuperscript{b} Data from 12 days after treatment removed from statistical analysis due to consistent residue non-detection.
\textsuperscript{c} Eastern Standard Time.
\textsuperscript{d} LSD (P < 0.05) for comparing days after treatment-by-time within a day interaction.
Table 5. Climatic conditions recorded for experiment 2 from 1 to 6 days after treatment.\textsuperscript{a,b}

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<th>TWD</th>
<th>RH</th>
<th>AT – DP</th>
<th>TFS</th>
<th>LW</th>
<th>RH</th>
<th>AT – DP</th>
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<th>LW</th>
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\textsuperscript{a} Abbreviations: DAT, days after treatment; TWD, time within a day; RH, relative humidity; AT, air temperature; DP, dew point; TFS, time from sunrise; LW, leaf wetness.

\textsuperscript{b} Climatic conditions recorded on site at the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC)
Table 6. Pearson correlation coefficients for experiment 2 quantifying the relationships between climatic parameters and dislodgeable 2,4-D following application on a simulated soccer field.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Climatic parameter</th>
<th>Dislodgeable 2,4-D as % of applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 DAT</td>
</tr>
<tr>
<td>Air temperature – dew point</td>
<td>-0.73\textsuperscript{c}</td>
</tr>
<tr>
<td>Leaf wetness</td>
<td>0.58\textsuperscript{**}</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>0.69\textsuperscript{c}</td>
</tr>
<tr>
<td>Time from sunrise</td>
<td>-0.82\textsuperscript{†}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviation: DAT, days after treatment.
\textsuperscript{b} Climatic conditions recorded on site at the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC).
\textsuperscript{c} †, ***, **, and * denote significance at P < 0.0001, 0.001, 0.01, and 0.05, respectively.
Figure 1. Percent dislodged following one soccer ball roll (3.7 m) from hybrid bermudagrass of the applied (2.1 kg 2,4-D ha\(^{-1}\)) at 14:00 (immediate) and 15:00 on 0 d after treatment (DAT), and from 5:00 to 13:00, 1 to 3 DAT. All sample collection times were Eastern Standard Time.
2,4-dimethylamine salt (2,4-D) is an herbicide commonly applied on athletic fields for broadleaf weed control that can dislodge from treated turfgrass. Dislodge potential is affected by numerous factors, including turfgrass canopy conditions. Building on previous research confirming herbicide-turfgrass dynamics can vary widely between species, field research was initiated in 2014 and 2015 in Raleigh, NC to quantify dislodgeable 2,4-D residue from dormant hybrid bermudagrass (Cynodon dactylon L. x C. transvaalensis) and hybrid bermudagrass overseeded with perennial ryegrass (Lolium perenne L.), which are common athletic field playing surfaces in subtropical climates. Additionally, dislodgeable 2,4-D was compared at AM (7:00 Eastern Standard Time) and PM (14:00) sample timings within a day. Samples collected from perennial ryegrass consistently resulted in greater 2,4-D dislodgment immediately after application (9.4 to 9.9% of applied) compared to dormant hybrid bermudagrass (2.3 to 2.9%), as well as at all AM compared to PM timings from 1 to 3 d after treatment (DAT; 0.4 to 6.3% compared to 0.1 to 0.8%). Dislodgeable 2,4-D did not differ across turfgrass species at PM sample collections, with ≤ 0.1% of the 2,4-D applied dislodged from 1 to 6 DAT, and 2,4-D detection did not occur at 12 and 24 DAT. In conclusion, dislodgeable 2,4-D from treated turfgrass can vary between species and over
short time-scales within a day. This information should be taken into account in human exposure risk assessments, as well as by turfgrass managers and athletic field event coordinators to minimize 2,4-D exposure.

1First, second, and fourth authors: Graduate Research Technician and Assistant Professor, respectively, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, North Carolina.

2Third and fifth authors: Associate Professor and Research Technician, respectively, Department of Plant Sciences, University of Tennessee, Knoxville, Tennessee.

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**Introduction**

Turfgrasses are used for a variety of societal purposes, including activities on athletic fields. The National Turfgrass Research Initiative reported in 2003 there were > 700,000 managed athletic fields in the US [1]. Maintaining a functional, safe playing surface for participants is the primary objective of athletic field managers, as poor footing conditions can lead to increased lower body injuries [2, 3]. In subtropical climates, warm-season turfgrass species such as bermudagrass (*Cynodon* spp.) are often overseeded during dormancy periods with a cool-season turfgrass to improve athletic field surface aesthetics and functionality [4]. Perennial ryegrass (*Lolium perenne* L.) is a cool-season species commonly utilized for overseeding because of its rapid germination (∼ 5 d), dark-green color and winter hardiness [5]. Regardless of the turfgrass species present on athletic fields, conditions can unfold that degrade playing surface quality and compromise playing surface safety via weed encroachment. Athletic field managers employ multiple weed control practices to alleviate this issue, including synthetic herbicide applications. One such herbicide athletic field managers utilize for selective broadleaf control is 2,4-dimethylamine salt (2,4-D), which is registered for use on cool- and warm-season turfgrasses [6]. Although research to date is inconclusive on human-carcinogenic effects from 2,4-D, it is a confirmed toxin to blood, kidney, and liver, as well as an eye irritant [6-10].

As with any pesticide, various transformation and transport processes ensue following application. Physicochemical properties specific to 2,4-D transport from the intended site include very high water solubility ($K_s = 796,000$ mg L$^{-1}$; 20° C) and a low soil organic carbon sorption coefficient ($K_{oc} = 20$ mL g$^{-1}$) [6, 11], which suggests 2,4-D may dislodge
from treated turfgrass vegetation. Nishioka et al. [12] reported 2,4-D consistently tracked into residences (10 total) from treated residential lawns up to 1 wk following application, with > 70% of total household loading attributed to children’s shoes and dogs. Jeffries et al. [13] reported 2,4-D dislodgment from hybrid bermudagrass fluctuated with daily canopy moisture conditions. More specifically, 4% of the applied was dislodged at 5:00:00 Eastern Standard Time (EST) 1 d after treatment (DAT), which declined to 0.1% by 13:00:00; however, dislodgment increased to 2.1% of the applied at 2 DAT–5:00:00 and similar trends persisted through 6 DAT.

Following pesticide dislodgement from treated turfgrass, various human exposure routes may occur. Specific to 2,4-D, nonoccupational human absorption occurs via dermal, as well as dietary and nondietary ingestion routes [12, 14-16]. Humans do not readily metabolize 2,4-D in the body, and it is ultimately lost via urine. Human exposure to 2,4-D is commonly confirmed via urine sampling, and a notable example of this is the 2001-2002 National Health and Nutrition Examination Survey, which reported urine-2,4-D detection occurrence in over 25% of the 546 children (ages 6 to 11 yr) evaluated [17].

Human pesticide exposure risk assessments are arduous endeavors that estimate the nature and probability of adverse health effects following exposure to contaminated environmental media [18]. Within the occupational and residential exposure test guidelines currently employed by the US Environmental Protection Agency (EPA), foliar dislodgeable residue dissipation tests (OPPTS 875.2100) are required for pesticide registration or re-registration [19]. The purpose of such tests is to quantify pesticide residue remaining on treated surfaces that can be dislodged through various processes on human skin/clothing or

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inhaled [21]. Within current US EPA protocols, experiment site selection considerations only reference climatic conditions representative of the intended use area [20]. Information pertaining to turfgrass species and management inputs may improve foliar dislodgeable residue dissipation tests, as growth characteristics vary widely between cool- and warm-season turfgrasses, and can affect pesticide-plant interactions [21]. Additionally, herbicide uptake, translocation and metabolism may differ across turfgrasses with relatively comparable growth characteristics (i.e. within cool- or warm-season turfgrasses) [22-24].

The objectives of this research were to quantify dislodgeable 2,4-D foliar residue between turfgrass species over two time scales, both within a day and over days. By doing so, 2,4-D human exposure assessments may be improved through incorporating more site-specific conditions. We hypothesized 2,4-D dislodgeability would vary between species and over the course of both time scales.

**Materials And Methods**

**Site Description**

Field experiments were initiated 9 April 2014 and 31 March 2015 (Lake Wheeler Turfgrass Field Laboratory, Raleigh, NC; Lat. 35°44'21.34” N, Long. 78°40'49.75” W) on a sandy clay loam soil with pH 6.4 and 1.9% organic matter w w⁻¹. Curtis Powell (Lake Wheeler Road Field Laboratory Superintendent, Raleigh, NC) granted permission to access experiment areas. Research was conducted on weed-free areas where 2,4-D had not been applied 2 yr preceding initiation. Prior to experiment initiation, vegetation and soil from research areas were analyzed to confirm nondetectable 2,4-D residue [25].
Research was conducted on established, dormant ‘Tifway’ hybrid bermudagrass alone, as well as areas overseeded (broadcasted at 976 kg pure live seed ha\(^{-1}\)) with ‘Carly’ perennial ryegrass in the fall prior to experiment initiation. Overseeding occurred on 12 September 2013 and 23 September 2014 for experiments initiated in 2014 and 2015, respectively. All areas were maintained as an athletic field surface with respect to fungicide/insecticide applications, nutrient applications (49 kg N ha\(^{-1}\) mo\(^{-1}\)), irrigation (provided to supplement rainfall) and mowing (1.9 cm height of cut; three events wk\(^{-1}\); clippings returned) [4]. Herbicides and plant growth regulators were not applied to experimental areas throughout the research.

**Experimental Design**

Research was conducted as a split plot, randomized complete block design with three replicates of a 2-by-2-by-6 factorial treatment arrangement each year. Main plots were split by turfgrass species (dormant hybrid bermudagrass or overseeded perennial ryegrass), with subplots combinations of 2 sample collection times within a day (7:00 or 14:00 EST) in each of 6 sample days (1, 2, 3, 6, 12 or 24 DAT). Dislodgeable residue samples were also collected immediately following application, and after a 1 h drying period on 0 DAT; however, these samples were statistically analyzed separately due to differing collection timings from other samples beyond 0 DAT. A nontreated check was included in all experimental blocks to ensure the trial area was not contaminated.

**Experiment Initiation**

One d prior to trial initiation, areas were mown (clippings collected) and irrigated to field capacity. Experimental areas were not irrigated or mown for 6 d following treatment.
Furthermore, areas were covered with plastic (HDX 6 Mil Clear Plastic; The Home Depot Corp., Atlanta, GA) suspended above the turfgrass canopy during rainfall events during this 6-d period. At experiment initiation, 2,4-D amine (Amine 400 2,4-D Weed Killer®; PBI/Gordon Corp., Kansas City, MO) was applied at 2.1 kg ai ha\(^{-1}\) to plots measuring 1.5 by 2.25 m (1 m alleys between blocks to enable sample collection without human-plot contact).

2,4-D was applied in accordance with local regulatory and manufacturer instructions regarding personal protective equipment, sprayer setup and use site. The selected application rate is labeled for use in turfgrass systems (sod production); however, is 20% higher than athletic field allowance. The increased application rate was required to ensure 2,4-D residue detection at 6 DAT, which we felt was a justifiable compromise to better elucidate the research variables of interest. Treatments were sprayed at 14:00:00 to allow the solution to dry on vegetation with \(\geq 5\) h of sunlight remaining that day. Applications were made with a hand-held CO\(_2\)-pressurized sprayer comprised of four 80015 XR VS flat-fan nozzles (TeeJet\(^{®}\) Flat-Fan Nozzles; spraying Systems Co., Wheaton, IL). The carrier volume selected (187 L ha\(^{-1}\) at 179 kPa) is the minimum stated on the label, creating the worst-case scenario for pesticide retention on the turfgrass canopy. To ensure 2,4-D was applied at the intended rate, cellulose-based sheets (387 cm\(^2\); Whatman™ 3 MM Chr Chromatography Paper, GE Healthcare Bio-Sciences, Pittsburgh, PA) were randomly placed throughout the trial area. Following 2,4-D spray application overtop sheets, residue was quantified by high performance liquid chromatography (HPLC) with a diode array detector (DAD) analysis.

Finally, air temperature, dew point, relative humidity and sunrise were logged throughout experiments (Table 1). Additionally, leaf wetness (Leaf Wetness Sensor; Decagon Devices
Inc., Pullman, WA) was measured with a flat-plate sensor facing north at a 45° angle from the ground surface at a 0.6 m height.

**Sample Collection**

*Dislodgeable 2,4-D*

Dislodgeable 2,4-D was quantified by rolling a soccer ball (Size 4; Franklin Sports Competition 100 Soccer Ball, Franklin Sports, Stoughton, MA) over a 9 m distance (four 2.25 m side-by-side rolls) within each sub-sub plot. 2,4-D dislodgment via this process was selected due to soccer’s rank as the most popular international sport coupled with the frequency of ball-to-turfgrass and subsequent ball-to-hand contacts inherent to play [26]. Ball roll distance was based off of a soccer ball rolling 50% of the recommended field length (18 m) for youth ages < 6 yr in the US [28]. The soccer ball was double-wrapped with a 5 by 120 cm cellulose-based sorbent strip (Scott Shop Towel™; Kimberly-Clark Corp., Neenah, WI). The soccer ball was mounted to a hand-held PVC apparatus designed such that the ball rotated end-over-end in the same direction as the sorbent strip, thus allowing for constant sorbent strip contact to the treated turfgrass surface (Fig 1; the individual in this figure has given written informed consent outlined in the PLOS consent form to publish these details).

While this is not representative of a ball roll when actively playing soccer, this measure was required to minimize variation in data and determine the maximum dislodgeable 2,4-D from turfgrass vegetation. Following ball roll, the entire sorbent strip was removed, placed in a unique glass jar (473 cm³) and stored at -12° C for subsequent extraction and HPLC-DAD analysis. Dislodgeable 2,4-D residue relative to the amount applied at trial initiation was calculated using the equation:
% dislodged of applied = \left[ \left( \frac{\text{BR} \, \mu g \, 2,4\text{-D cm}^{-2}}{20.9 \, \mu g \, 2,4\text{-D cm}^{-2}} \right) \times 100 \right]

where BR represents 2,4-D residue recovered from ball roll samples relative to the 2,4-D application rate (20.9 \, \mu g \, 2,4\text{-D cm}^{-2}).

**Turfgrass Vegetation Residue**

The quantity of 2,4-D within or on the surface of turfgrass vegetation (i.e., in/on) was quantified at all sample collections to: 1) quantify vegetation spray interception across species; and 2) characterize 2,4-D dissipation in/on dormant hybrid bermudagrass and perennial ryegrass vegetation over time. This was done by collecting a core (10.8 cm diam; 92 cm$^2$) such that sampling equipment did not contact aboveground vegetation. Following collection, all samples were frozen, turfgrass vegetation was harvested, weighed, processed [1.7 mm (Fitzmill Homoloid Model JT 6; Fitzpatrick Co., Elmhurst, IL)] and stored at -12$^o$C until extraction and residue analysis. 2,4-D residue in/on turfgrass vegetation was calculated as a percent of the amount applied at trial initiation was calculated with the equation:

% of applied = \left[ \left( \frac{\text{AV} \, \mu g \, 2,4\text{-D cm}^{-2}}{20.9 \, \mu g \, 2,4\text{-D cm}^{-2}} \right) \times 100 \right]

where AV represents 2,4-D residue recovered from aboveground vegetation as a percent of the 2,4-D application rate (20.9 \, \mu g \, cm$^{-2}$).
**Residue Analyses**

2,4-D residue was quantified with HPLC-DAD (Agilent-1260 Infinity; Agilent Technologies, Inc., Wilmington, DE) methodology. Details pertaining to sample preparation, extraction and cleanup, as well as analytical parameters are provided in Jeffries et al. [13]. In summary, limits of detection and quantification were 0.3 and 1.0 mg L\(^{-1}\), respectively, while maintaining the signal to noise ratio at 3:1. 2,4-D residue was quantified using peak area measurements (OpenLAB CDS ChemStation, Version C.01.04; Agilent Technologies, Inc., Wilmington, DE) and concentrations above the calibration curve were diluted and re-injected for analysis. Fortification recovery checks for sorbent strips and vegetation matrices ranged from 93 to 103 and 90 to 96%, respectively, across all analyses conducted in the presented research. Lastly, application recovery check sheets determined 2,4-D was applied at 95% of the intended rate across years.

**Statistical Analyses**

Statistical analyses were conducted by ANOVA (P = 0.05) using MIXED procedures in SAS (Statistical Analysis Software®; Version 9.2; SAS Institute, Inc., Cary, NC). Turfgrass species and sample collection timings were considered fixed effects, while year and replicate were considered random as described by Carmer et al. [28]. Main effects and their interactions are presented accordingly, with precedent given to significant interactions of increasing magnitude [29]. Means were separated according to Fisher’s protected LSD (P < 0.05) and Pearson correlation coefficients (P = 0.05) were determined to quantify the relationships between selected climatic conditions with dislodgeable 2,4-D plant residue.
Results And Discussion

2,4-D Dislodgeability

2,4-D residue detection did not occur in dislodgment samples collected 12 and 24 DAT; therefore, these data were excluded from statistical analysis. Nondetection at 12 and 24 DAT may be due in part to irrigation or precipitation inputs following 6 DAT sample collection, as previous research has shown dislodgment of the highly water soluble herbicide is reduced following water inputs [13, 30]. ANOVA revealed significant interactions for all sources of variation including year; therefore, data were sorted by year and presented accordingly. Additionally, ANOVA revealed a significant turfgrass-by-sample collection time within a day interaction from 0 to 6 DAT, which is presented. Across years, greater 2,4-D dislodgment occurred from perennial ryegrass compared to dormant hybrid bermudagrass. Within perennial ryegrass, greater 2,4-D dislodgment occurred at AM compared to PM sample collections. Samples collected immediately after application resulted in > three-fold greater dislodgment in perennial ryegrass (9.4 to 9.9% of applied across runs) compared to dormant hybrid bermudagrass (2.3 to 2.9%), while a 1 h dry time decreased dislodge to ≤ 0.5% across species (Table 2). At 1 DAT, 3.7 and 6.3% of the applied 2,4-D dislodged from perennial ryegrass in 2014 and 2015, respectively, while less dislodged from dormant hybrid bermudagrass (0.6 to 0.8%). Across species in 2014, greater 2,4-D dislodgment occurred at 1 DAT in AM (0.6 to 3.7% of applied) compared to PM (0.1%) sample collections, while this was only true for perennial ryegrass in 2015 (6.3 and 0.1% of applied in AM and PM, respectively). At 2 and 3 DAT across years, greater 2,4-D dislodgment occurred from perennial ryegrass than dormant hybrid bermudagrass in the AM (0.4 to 4.1 and ≤ 0.6% of
applied, respectively). Additionally, 2,4-D dislodgment declined to \( \leq 0.1\% \) of the applied in the PM across turfgrass species. Although statistical separation is not permissible at 6 DAT, 2,4-D detection occurred for both turfgrass species and sample collection times in 2014 (\( \leq 1.7\% \) of applied), and only for perennial ryegrass–AM in 2015 (0.2%).

Although statistical separation is not permissible as presented, dislodged 2,4-D from perennial ryegrass consistently increased from PM sampling on a given day to AM sampling the subsequent day (Fig 2.). This trend also occurred in dormant hybrid bermudagrass from 0 DAT–1 h to 1 DAT–AM, and sporadically through 6 DAT. This may be due to 2,4-D re-suspension overnight on turfgrass vegetation as conditions become more favorable for moisture development. Jeffries et al. [13] reported similar trends from actively growing hybrid bermudagrass, as 2,4-D dislodged 1 DAT–13:00 (0.1% of applied) increased at 2 DAT–5:00 (2.1%)

**2,4-D Persistence in Turfgrass Vegetation**

ANOVA revealed significant interactions for all sources of variation including year; therefore, data were sorted by year. 2,4-D persistence varied between species at various DAT, and did not vary between sample collections in each day within species; therefore, the main effect of turfgrass species is presented and discussion is focused on highlighting factors that may have contributed to varying dislodgeable 2,4-D results between perennial ryegrass and dormant hybrid bermudagrass. When statistical separation occurred between turfgrass species, 2,4-D persistence in perennial ryegrass turfgrass vegetation outranked dormant hybrid bermudagrass. Although not statistically different in 2014, samples collected at 0 DAT suggest 2,4-D spray interception varied between species, as 75 to 96% of the applied
was recovered in perennial ryegrass vegetation compared to 52 to 82% in hybrid bermudagrass (Table 3). This may be due in part to morphological differences between actively growing perennial ryegrass and dormant hybrid bermudagrass, which this study is unable to confirm. Additionally, this may be due in part to increased aboveground biomass in a hybrid dormant bermudagrass area overseeded with perennial ryegrass compared to solely a dormant hybrid bermudagrass area. Averaged over years, fresh aboveground biomass core\(^{-1}\) was 4.9 and 9.3 g from dormant hybrid bermudagrass and perennial ryegrass, respectively (data not shown). In general, greater vegetation-spray interception occurred in 2015, which may also be attributed to greater aboveground biomass between years. Dormant hybrid bermudagrass and perennial ryegrass fresh aboveground biomass core\(^{-1}\) was 206 and 150% greater in 2015 than 2014, respectively (data not shown).

Relative to respective 0 DAT data, 2,4-D persisted similarly in/on turfgrass vegetation across species, as persistence declined 25 and 27% in dormant hybrid bermudagrass and perennial ryegrass in 2014 and 43 and 47% in 2015, respectively, through 6 DAT. Similar dissipation rates between dormant hybrid bermudagrass and actively growing perennial ryegrass were not expected, and the presented research cannot elucidate the reason for this occurrence; however, the amine 2,4-D formulation evaluated in this research has poor foliar uptake compared to other formulations, which application and management practices through 6 DAT favored [31]. Additionally, Weintraub et al. [32] reported 2,4-D was metabolized in dormant cherry tree (Prunus avium) buds, as > 90% \(^{14}\)C-2,4-D dissipation occurred over 3 to 5 mo dormancy periods. The dramatic 2,4-D residue decline in turfgrass vegetation from 6 to 12 DAT is likely due to irrigation or precipitation
inputs coupled with mowing events during this time period. Finally, 2,4-D detection in/on turfgrass vegetation occurred consistently across years and turfgrasses at 24 DAT, suggesting management practices to minimize off-target transport should be employed for at least this duration following application.

**Climatic Condition Correlations with Dislodgeable 2,4-D**

Climatic data from 0 to 6 DAT suggest dislodgeable 2,4-D may be influenced by conditions favoring turfgrass canopy moisture. Relative humidity is a measurement of atmospheric moisture relative to saturated air, with increasing values suggesting increased atmospheric moisture [33]. Dew point is the air temperature below which moisture in the atmosphere condenses, and as difference in relative humidity and dew point decrease, dew formation becomes more likely [13, 34]. Maximum dew formation on plant canopies has previously been reported to occur at, or just after sunrise [13, 35, 36]. Although turfgrass canopy moisture is not solely influenced by these climatic parameters, previous research has shown they are correlated with 2,4-D dislodge from treated vegetation [13]. Jeffries et al. [13] reported correlations between dislodgeable 2,4-D at 1 DAT and air temperature – dew point, leaf wetness, relative humidity and time from sunrise were -0.73 (P < 0.0001), 0.58 (P < 0.01), 0.69 (P < 0.0001) and -0.82 (P < 0.0001), respectively.

Pooled over data from 1 to 6 DAT, 2,4-D dislodgeability from dormant hybrid bermudagrass was strongly correlated (r ≥ 0.7) with leaf wetness in both years (r = 0.85 to 0.94) and relative humidity in 2015 (r = 0.73), suggesting dislodgment increased as leaf wetness and atmospheric moisture increased (Table 4). These variables were also strongly correlated with 2,4-D dislodgment from perennial rye grass (leaf wetness r = 0.82 to 0.96;
relative humidity $r = 0.71$ to 0.8). Additionally, strong negative correlations were detected between 2,4-D dislodgment from perennial ryegrass and air temperature – dew point in both years ($r = -0.77$ to -0.79) and time from sunrise in 2014 ($r = -0.81$), suggesting dislodgment increased as air temperature approached dew point and time from sunrise decreased.

Specific to perennial ryegrass, dislodgeable 2,4-D at AM samplings declined from 1 to 2 DAT, and increased from 2 to 3 DAT in both years, which may be explained in part by climatic conditions. More specifically, 2 DAT–AM had lower relative humidity (60 and 61%), the largest difference between air temperature and dew point (6.6 and 7.2° C) and lowest leaf wetness (273 mV) than other AM sample collections from 1 to 6 DAT (excluding 2015–6 DAT; Table 1). Wichink Kruit et al. [37] reported a 71% relative humidity threshold was required for moisture development on grass canopies, which likely varies based on site-specific conditions; however, increased relative humidity generally aligned with increasing leaf wetness measurements through 3 DAT in our research.

**Research Implications**

Data from the presented research were used to predict daily human dermal 2,4-D exposure as calculated by US EPA for post-application exposure for physical activities on turfgrass using the equation:

$$[3] \ E = TTR_t \times CF1 \times TC \times ET$$

where $E =$ exposure (mg d⁻¹); $TTR_t =$ turf transferable residue on day $t$ (X µg 2,4-D cm²); $CF1 =$ unit conversion factor (0.001 mg µg⁻¹); $TC =$ transfer coefficient (49,000 cm² hr⁻¹; 1 to 2 yr children); and $ET =$ exposure time (1.5 hr d⁻¹; 1 to 2 yr children) in the algorithm [13,
The algorithm, as well as TC and ET coefficients were obtained from the US EPA risk assessment, while TTR₁ (X μg 2,4-D cm⁻²) corresponds to the dislodgment data in the presented research [13, 40]. Comparing the highest dislodgeable residue values from each turfgrass species after the day of application (2015–1 DAT–AM), it was calculated that a human could potentially be exposed to 12 and 96 mg 2,4-D d⁻¹ from one soccer ball roll over a 0.19 m² area of dormant hybrid bermudagrass and perennial ryegrass, respectively. Adjusting this for the 10% 2,4-D dermal absorption rate utilized in the US EPA risk assessment results in 1.2 and 9.6 mg 2,4-D d⁻¹ from dormant hybrid bermudagrass and perennial ryegrass, respectively [6, 13, 38]. Using the risk assessment value for short-term (30 d) human dermal exposure of 25 mg kg⁻¹ d⁻¹, it was determined that an average 1 to 2 yr old child (11 kg) may be dermally exposed to 275 mg 2,4-D d⁻¹ without adverse effect [6, 13]. By calculating our observed maximum daily exposure (1.2 and 9.6 mg 2,4-D d⁻¹) as a percent of the short-term daily dermal exposure allowance without adverse effect (275 mg 2,4-D d⁻¹), it was determined that 2,4-D dislodged from one ball roll equaled 0.4 and 3.5% of this limit from dormant hybrid bermudagrass and perennial ryegrass, respectively. Across turfgrass species, all PM sample collections from 1 to 6 DAT resulted in ≤ 0.06% of the daily limit. It should be noted again that the 2,4-D application rate in the presented research was 20% greater than current label allowances on athletic fields, which overestimates in situ exposure with this algorithm; however, the area covered by one ball roll in this research equals 0.07% of the area of the smallest children’s (U6) soccer field (14 by 18 m) recommended by the US Youth Soccer Organization [13, 27].
The presented research is not intended to detract from the approach current regulatory agencies use to estimate human 2,4-D exposure. Instead, the intention is to provide information to improve such efforts. To our knowledge, previous research efforts have not compared 2,4-D dislodgment between turfgrass species simultaneously, and excluding Jeffries et al. [13], reports have either not specifically stated when sample collections occurred within a day or were collected after 10:00:00 EST (30, 39, 40). For example, the sole field experiment evaluating human 2,4-D dermal exposure from treated turfgrass in the 2005 re-registration package collected samples at 12:30:00 EST (0 and 1 DAT) [6, 30].

Results from this research suggest 2,4-D human exposure assessments may be improved by including additional sample collections at earlier and later times of day when canopy moisture is more likely to be present. Additionally, the aforementioned experiment does not specifically state the turfgrass species in the report, which this research confirms is an influencing factor on 2,4-D dislodgeability [30].

Conclusions

This research built on preceding efforts evaluating dislodgeable 2,4-D from treated turfgrass. Our experiment quantified dislodgeable 2,4-D from two common athletic field turfgrass species via soccer ball roll, a process common to the most popular international sport. Results indicate 2,4-D can dislodge from dormant hybrid bermudagrass and perennial ryegrass up to 6 DAT when water inputs and mowing practices are not employed following application. Ultimately, 2,4-D’s physicochemical properties coupled with varying canopy dynamics between turfgrass species resulted in differing dislodgment between species and
sample collection times within a day. More specifically, perennial ryegrass possessed more aboveground biomass, which resulted in greater 2,4-D spray-vegetation interception. Coupling this with increased morning canopy moisture at samplings and 2,4-D’s very high water solubility resulted in maximum 2,4-D dislodgment with perennial ryegrass–AM samplings. Excluding the day of application, ≤ 0.1% of the applied 2,4-D was dislodged at PM samplings across species, suggesting human activity on recently treated fields is safe when canopy moisture is not present.

In conclusion, dislodgeable 2,4-D on athletic fields can vary depending on turfgrass canopy characteristics, and information pertaining to species and conditions favoring canopy moisture presence should be included in human exposure risk assessments. Based off 2,4-D dislodgeable residue measured via soccer ball roll in this research, a relatively nonaggressive approach compared to other athletic processes, these data suggest turfgrass managers and athletic field event schedulers should coordinate 2,4-D application to avoid human activity when canopy moisture is present for at least 6 d following treatment; however, this period will likely vary depending on site-specific conditions and management practices. Future research should investigate similar research objectives with additional pesticides, dislodge methods and turfgrass species, as well as the effect of sprayer setup, surfactant tank-mixes and mowing practices to reduce dislodgeable 2,4-D residue from turfgrass.
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<td>80</td>
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<td>10</td>
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<td>81</td>
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<td>266</td>
<td>30</td>
<td>17.6</td>
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<td>269</td>
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<td>33</td>
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<td>271</td>
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<td>7:00</td>
<td>80</td>
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<td>272</td>
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<td>10.0</td>
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<td>274</td>
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<td>21.1</td>
<td>424</td>
<td>267</td>
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^a Abbreviations: DAT, days after treatment; TWD, time within a day; RH, relative humidity; AT, air temperature; DP, dew point; TFS, time from sunrise; LW, leaf wetness.

^b Climatic conditions recorded on site at the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC)

^c Eastern Standard Time.
Table 2. The effect of irrigation on dislodgeable 2,4-D relative to the applied at trial initiation.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Turfgrass</th>
<th>0 DAT</th>
<th>1 DAT</th>
<th>2 DAT</th>
<th>3 DAT</th>
<th>6 DAT</th>
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<tr>
<td></td>
<td>0 h\textsuperscript{c}</td>
<td>1 h AM</td>
<td>AM PM</td>
<td>AM PM</td>
<td>AM PM</td>
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<tr>
<td>Hybrid bermudagrass</td>
<td>2.3</td>
<td>0.1</td>
<td>0.6</td>
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<td>&lt; 0.1</td>
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<tr>
<td>Perennial ryegrass</td>
<td>9.9</td>
<td>0.5</td>
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<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>LSD\textsubscript{0.05}</td>
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<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Turfgrass</th>
<th>0 DAT</th>
<th>1 DAT</th>
<th>2 DAT</th>
<th>3 DAT</th>
<th>6 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>1 h AM</td>
<td>AM PM</td>
<td>AM PM</td>
<td>AM PM</td>
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<tr>
<td>Hybrid bermudagrass</td>
<td>2.9</td>
<td>&lt; 0.1</td>
<td>0.8</td>
<td>&lt; 0.1</td>
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<td>Perennial ryegrass</td>
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<td>6.3</td>
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<tr>
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<td>0.3</td>
<td>0.1</td>
<td>1.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: DAT, days after treatment; AM, 7:00 EST; PM, 14:00 EST; ND, non-detection; NS, non-significant.
\textsuperscript{b} Data from 12 and 24 days after treatment removed from statistical analysis due to consistent residue non-detection.
\textsuperscript{c} Dislodge samples collected immediately and 1 hour following 2,4-D application.
\textsuperscript{d} LSD (P < 0.05) for comparing turfgrass-by-sample time within a day interaction.
Table 3. 2,4-D persistence in dormant hybrid bermudagrass (*C. dactylon* x *C. transvaalensis*) and non-dormant perennial ryegrass (*Lolium perenne*) aboveground vegetation.\textsuperscript{a,b}

<table>
<thead>
<tr>
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<th>0 DAT</th>
<th>1 DAT</th>
<th>2 DAT</th>
<th>3 DAT</th>
<th>6 DAT</th>
<th>12 DAT</th>
<th>24 DAT</th>
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<tbody>
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<td>52</td>
<td>54</td>
<td>50</td>
<td>38</td>
<td>39</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>75</td>
<td>69</td>
<td>68</td>
<td>60</td>
<td>55</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>LSD\textsubscript{0.05}\textsuperscript{c}</td>
<td>NS</td>
<td>NS</td>
<td>17</td>
<td>NS</td>
<td>NS</td>
<td>2</td>
<td>NS</td>
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2015

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<th>1 DAT</th>
<th>2 DAT</th>
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<th>6 DAT</th>
<th>12 DAT</th>
<th>24 DAT</th>
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<tbody>
<tr>
<td>Hybrid bermudagrass</td>
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<td>61</td>
<td>63</td>
<td>62</td>
<td>47</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>96</td>
<td>83</td>
<td>86</td>
<td>76</td>
<td>51</td>
<td>5</td>
<td>2</td>
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\textsuperscript{a} Abbreviations: DAT, days after treatment; NS, non-significant.

\textsuperscript{b} Irrigation/precipitation and mowing did not occur from 0 to 6 day after treatment sample collections.

\textsuperscript{d} LSD (P < 0.05) for comparing turfgrass within a day after treatment.
Table 4. Pearson correlation coefficients quantifying the relationships between climatic parameters and dislodgeable 2,4-D dormant hybrid bermudagrass (\textit{C. dactylon} x \textit{C. transvaalensis}) and non-dormant perennial ryegrass (\textit{Lolium perenne}).\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Climatic parameter</th>
<th>Dislodgeable 2,4-D as % of applied</th>
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<th></th>
<th></th>
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<td></td>
<td>Bermuda</td>
<td>Rye</td>
<td>Bermuda</td>
<td>Rye</td>
</tr>
<tr>
<td>Air temperature – dew point</td>
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<td>-0.79***</td>
<td>-0.69***</td>
<td>-0.77†</td>
</tr>
<tr>
<td>Leaf wetness</td>
<td>0.85†</td>
<td>0.82†</td>
<td>0.94†</td>
<td>0.96†</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>0.42*</td>
<td>0.71†</td>
<td>0.73†</td>
<td>0.80†</td>
</tr>
<tr>
<td>Time from sunrise</td>
<td>-0.40</td>
<td>-0.81†</td>
<td>-0.53**</td>
<td>-0.69**</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Data pooled over 1 through 6 day after treatment sample collections.

\textsuperscript{b} Climatic conditions recorded on site at the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC).

\textsuperscript{c} †, ***, **, and * denote significance at P < 0.0001, 0.001, 0.01, and 0.05, respectively.
Figure 1. Frame constructed of PVC (5 cm inner diameter) and lag bolts to mount a soccer ball, which allows for a consistent end-over-end ball roll and constant sorbent strip-to-turfgrass contact.
Figure 2. Dislodged 2,4-D following one soccer ball roll (0.19 m$^2$) over turfgrass as a percent of the application rate (2.1 kg ai ha$^{-1}$). Sample collections occurred at 0 hour (14:00) and 1 hour (15:00) after application on 0 day after treatment (DAT), and 7:00 and 14:00 from at AM and PM sample collections, respectively.
CHAPTER 4: 2,4-D AND AZOXYSTROBIN TOLERANCE AND BIOREMOVAL CAPACITY OF AQUATIC PLANTS NATIVE TO THE SOUTHEAST UNITED STATES.

Formatted for publication in the International Journal of Phytoremediation.

Matthew D. Jeffries¹*, Fred H. Yelverton¹, and Travis W. Gannon¹

Despite concerted efforts by turfgrass managers to prevent pesticide transfer from the intended site, they can relocate into adjacent surface water bodies and adversely effect aquatic ecosystems. Therefore, research was conducted to quantify the pesticide bioremoval capacity of aquatic plants suitable for surface water bodies neighboring managed turfgrass systems in the southeast United States. Greenhouse experiments quantified 2,4-D (herbicide) and azoxystrobin (fungicide), two commonly applied pesticides in turfgrass systems, removal from pond water in containers comprised of arrow arum (*Peltandra virginica* L.), pickerelweed (*Pontederia cordata* L.), or Virginia iris (*Iris virginica* L.). Pesticide residue in water was adjusted to 5 mg L⁻¹ at initiation, and water samples were collected 0, 2, 4, 7, 14, and 28 days after treatment (DAT) for residue analysis. Additionally, plants were destructively harvested and sectioned into above- and below-soil surface portions at 14 and 28 DAT for residue analysis. Results suggest plants more readily removed azoxystrobin from water than 2,4-D. This may be due in part to compromised plant growth via herbicide injury, which was observed across all species. Overall, Virginia iris reduced pesticide residue in water more than arrow arum or pickerelweed at 28 DAT. Finally, pesticide residue in plant biomass persistence varied across between species, which may have
implications for aquatic plant management. Information from this research will help select plant species that are best suited to mitigate adverse effects from neighboring pesticide-treated areas, including turfgrass systems.

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Keywords: macrophyte, pesticide phytoremediation, stormwater wetland, surface water.
Introduction

When appropriately managed, turfgrass systems provide numerous beneficial environmental impacts such as carbon dioxide sequestration, soil erosion and dust control, enhanced heat dissipation, and noise abatement.\textsuperscript{1} However, misuse of fertilizer, pesticides, and irrigation inputs have been found to cause adverse effects to surface and groundwater sources.\textsuperscript{2,3} This is well illustrated in a national survey of surface water pesticide contamination reported by the United States Geological Survey, which found $> 1$ pesticide/metabolites in $> 90\%$ of the 186 test sites.\textsuperscript{4} Results from this research are concerning for aquatic ecosystem health in urban areas, as detection of pesticide exceedances of an aquatic life benchmark was $\approx 25\%$ greater in this land use than agricultural, undeveloped, or mixed-use sites.\textsuperscript{4}

2,4-D dimethylamine salt (2,4-D) is a synthetic auxin herbicide widely used for postemergent dicotyledonous weed control in turfgrass systems.\textsuperscript{5} Borges et al.\textsuperscript{6} reported 28\% of annual 2,4-D use in the United States (22 million kg; 1992 to 2000) was in managed turfgrass systems. Azoxystrobin (methyl(E)-2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) is a systemic strobilurin fungicide used for controlling turfgrass diseases, and although sales were not specifically for turfgrass, it was the world’s largest gross selling fungicide in 2008 ($900$ million US).\textsuperscript{7} Both pesticides are currently registered in turfgrass systems including athletic fields, commercial/residential lawns, golf courses, and parks, and can be applied to most turfgrass species managed in the United States.\textsuperscript{8,9}
2,4-D and azoxystrobin are classified by the United States Environmental Protection Agency as mobile and moderately mobile in soils, respectively, suggesting they can move from the intended site.\textsuperscript{5,10} To an extent, this has been confirmed in turfgrass systems through previous research. Ambrust and Peeler\textsuperscript{11} reported 2.6\% of the applied 2,4-D was detected in runoff collected from hybrid bermudagrass \textit{(Cynodon dactylon L. x C. transvaalensis)} on a 5\% slope. Models created by Haith and Rossi\textsuperscript{12} simulating runoff of 15 common turfgrass pesticides in the Northeast United States determined in rare events (1 in 10 year rainfall event simulation), azoxystrobin runoff exceeded LC\textsubscript{50}’s for rainbow trout \textit{(Salmo gairdneri}; 0.47 mg L\textsuperscript{-1}) and water flea \textit{(Daphnia magna}; 0.259 mg L\textsuperscript{-1}). An additional transfer route was identified by Jeffries et al.\textsuperscript{13}, who reported 2,4-D and azoxystrobin residue detection in hybrid bermudagrass \textit{(Cynodon dactylon L. x C. transvaalensis)} and tall fescue \textit{[Lolium arundinaceum (Schreb.) Darbysh.]} clippings collected 32 DAT. Furthermore, the authors reported 10 to 41\% of residue in clippings released into pond water over 2 days.\textsuperscript{13} 2,4-D and azoxystrobin relocation into surface water bodies poses ecotoxicological concerns, as they are highly toxic to various aquatic organisms (Table 1).

Riparian buffer zones and stormwater wetlands are commonly established in areas neighboring turfgrass systems to mitigate off-target pesticide transport. Design and management specifications for these systems are immense, and beyond the scope of this manuscript; however, it should be noted that despite previous reports of bioremoval capacity differing between plant species in these systems, plant selection recommendations commonly do not incorporate this information for in situ practice.\textsuperscript{14,15} Rice et al.\textsuperscript{16} reported hornwort \textit{(Ceratophyllum demersum)}, American elodea \textit{(Elodea canadensis)}, and common duckweed
(Lemna minor) all reduced metolachlor and atrazine, two herbicides, residues in water (38 to
60% less residue than non-planted containers). However, hornwort and American elodea
reduced residue in water (1 to 4% of applied remaining at 16 DAT) more than common
duckweed (23% of applied remaining at 16 DAT). Interestingly, from a separate
experiment, common duckweed was the best suited plant species [compared to Canadian
waterweed (Elodea canadensis Michx.) and Carolina fanwort (Cabomba aquatica A. Gray)]
for copper sulfate (fungicide), flazasulfuron (herbicide), and dimethomorph (fungicide)
residue removal in water.

Plant selection considerations for constructed pesticide mitigation areas typically
focus on promoting diversity, and selecting non-invasive, native species that are well adapted
for the specific growth setting of interest. While these are very sound principles, plant
selection for mitigation areas that neighbor turfgrass systems may pose unique challenges.
For example, an upright plant growth habit may be undesirable on golf courses due to
playability issues. Additionally, plants that pose allergic and tactile injury concerns are not
advisable for public health concerns. Incorporating these additional considerations, the
objective of this research was to evaluate the pesticide-water bioremoval potential of aquatic
plant species native to the southeast United States that are well suited for establishment in
areas neighboring turfgrass systems.
Materials And Methods

Research Overview

Greenhouse research (Method Road Greenhouses, Raleigh, NC) was initiated to evaluate the pesticide-water bioremoval capacities of various aquatic plant species. Plant species included arrow arum (*Peltandra virginica* L.), pickerelweed (*Pontederia cordata* L.), and Virginia iris (*Iris virginica* L.), which are all low-growing, perennial wetland plants, native to the southeast United States, and are commonly recommended to plant in constructed pesticide mitigation areas.¹³,²⁰,²¹ Plants were established in unique plastic containers (729 cm² surface area; 3,000 cm³ volume) in sandy soil (98% sand-sized particles) with 2% organic matter w w⁻¹. Soil organic matter content was confirmed through loss by ignition (500°C for 12 h). Greenhouse conditions were set to provide 32/27°C day/night temperatures and a 14 h day length. Plants were grown for 2 mo under greenhouse conditions in locally collected pond water with confirmed non-detectable 2,4-D and azoxystrobin residues. Prior to experiment initiation, plant containers were emptied, cleaned, and refilled with 3 L fresh pond water. Plants were placed back into containers 12 h following these steps to allow water temperatures to acclimate with greenhouse conditions. Finally, pesticide treatments were applied 12 h following plant re-submersion.

Pesticides included 2,4-D (Amine 400 2,4-D Weed Killer®, PBI Gordon Corp., Kansas City, MO) and azoxystrobin (Heritage TL®, Syngenta Crop Protection, Inc., Greensboro, NC) applied at 15 mg ai plant container⁻¹ to create a 5 mg L⁻¹ pesticide concentration at 0 DAT. This rate was chosen to ensure residue detection over the course of this research. Applications were made by syringing 20 mL of a stock pesticide solution over
the water surface. Water within a respective container was agitated with disposable plastic stirrers within 1 h following application. Following pesticide treatment, water was stirred and containers were re-randomized every other day to minimize greenhouse microclimate effects the duration of the experiment. Water samples were collected 0, 2, 4, 7, 14, and 28 DAT. At 14 and 28 DAT, destructive sampling occurred to quantify pesticide residue in above- and below-soil surface plant structures. Pesticide residue data were converted to a percent of the applied at experiment initiation with the equation:

\[
\text{Eq. [1]} \% \text{ of applied} = \{(RA \times HM) / TR \times 100\}
\]

where RA, HM and AR represent residue analysis (mg ai g\(^{-1}\) biomass), harvest mass (g\(^{-1}\) biomass) and treatment rate (15 mg ai), respectively. Additionally, water residue data were also converted to a percent reduction from the non-planted control container at a respective sample timing with the equation:

\[
\text{Eq. [2]} \% \text{ reduction} = \{[1 – (mg ai PC / mg ai NPC)] \times 100\}
\]

where PC and NPC represent planted container and non-planted container, respectively.

Finally, visual plant injury was estimated at 7, 14, and 28 DAT based on a 0 to 100% scale (0 = no injury; 100 = complete plant death).
Residue Analyses

2,4-D and azoxystrobin residues were quantified with high performance liquid chromatography–diode array detector methodology (Agilent-1260 Infinity; Agilent Technologies, Inc., Wilmington, DE). Details pertaining to sample preparation, extraction and cleanup, as well as analytical parameters are provided in Jeffries et al. In summary, 2,4-D limits of detection and quantification were 0.3 and 1.0 mg L\(^{-1}\), respectively, while maintaining the signal to noise ratio at 3:1. Azoxystrobin limits of detection and quantification were 0.05 and 0.25 mg L\(^{-1}\), respectively. Pesticide residue was quantified using peak area measurements (OpenLAB CDS ChemStation, Version C.01.04; Agilent Technologies, Inc., Wilmington, DE), and concentrations above the calibration curve were diluted and re-injected for analysis. Fortification recovery checks for water and vegetation matrices ranged from 95 to 102 and 88 to 99\%, respectively, across all analyses conducted in the presented research.

Experimental Design and Statistical Analysis

Experiments evaluated a 2-by-3 factorial treatment arrangement. Factorial levels included two pesticides (2,4-D or azoxystrobin) and three aquatic plant species (arrow arum, pickerelweed, or Virginia iris). Four replicates of each pesticide–aquatic plant combination were arranged in a randomized complete block design to account for variable growth conditions within the greenhouse. Statistical analysis was conducted by ANOVA (P = 0.05) using general linear models in SAS (Statistical Analysis Software\textsuperscript{®}, Version 9.2, SAS Institute, Inc., Cary, NC). Plant species and sample timing were considered fixed effects. Main effects and their interactions are presented accordingly, with precedent given to
significant interactions of increasing magnitude.\textsuperscript{22} Means were separated according to Fisher’s protected LSD (P < 0.05).

\textbf{Results and Discussion}

\textit{Pesticide Residue in Water}

A significant plant species-by-water sample collection timing interaction was detected for both pesticides from 7 to 28 DAT. Therefore, data were separated by DAT, and plant species are compared within sample collection timing. At 7 DAT, plant species did not reduce 2,4-D or azoxystrobin residue in water (4.9 to 5.6 mg L\textsuperscript{-1}) compared to non-planted containers (5.1 to 5.6 mg L\textsuperscript{-1}) (Table 2). However, all plant species reduced pesticide residue in water at 14 and 28 DAT. 2,4-D residue in non-planted containers from 14 to 28 DAT was 4.3 and 4.1 mg L\textsuperscript{-1}, respectively, while plants reduced this to 2.0 to 3.7 at 14 DAT, and 0.5 to 1.8 mg L\textsuperscript{-1} at 28 DAT. A similar, but more pronounced reduction occurred for azoxystrobin. Azoxystrobin residue in non-planted containers from 14 to 28 DAT 4.6 and 4.5 mg L\textsuperscript{-1}, respectively, while plants reduced this to 1.0 to 2.7 at 14 DAT, and were < 0.1 mg L\textsuperscript{-1} at 28 DAT. When statistical separation between plants occurred, residue concentrations in water ranked arrow arum > pickerelweed > Virginia iris, with Virginia iris showing the most promise for phytoremediation.

While data confirm aquatic plants reduced pesticide residue in water at 14 and 28 DAT, solely presenting concentrations understates the bioremoval capacity due to differing water demands between planted and non-planted containers. Chen et al.\textsuperscript{23} reported a similar observation from a conceptually comparable bioremoval experiment, where superior nitrogen
and phosphorus reduction in water by canna (*Canna x generalis* Bailey; 98.7% N and 91.8% P removed) was in part due to a four-fold higher daily water demand compared to iris (*Iris pseudacorus*; 31.6% N and 38.5% P removed) and arrow arum (31.5% N and 26.3% P removed). Therefore, pesticide residues in water data were adjusted with the total volume of water container$^{-1}$ to calculate residue reduction from a planted container relative to non-planted container within a replicate (Table 3).

Pesticide reduction in water followed similar trends across compounds. Across pesticides at 7 DAT, bioremoval from Virginia iris (27 to 45% reduction) outranked pickerelweed (azoxystrobin only; 37% reduction) and arrow arum (15 to 21% reduction) (Table 4). At 14 DAT, plants similarly reduced 2,4-D (76 to 78%), which was also true for azoxystrobin at 14 DAT (95 to 99%) and 28 DAT (98 to 99%). Virginia iris reduced 2,4-D in water (92%) at 28 DAT more than arrow arum or pickerelweed (74 to 77%). Overall, the evaluated aquatic plant species reduced pesticide residue in water > 75% relative to non-planted containers by 14 DAT, which supports the use of implementing pesticide phytoremediation strategies in areas neighboring turfgrass systems.$^{14,15,18,19}$

**Pesticide Residue in Plants**

A significant plant species-by-plant harvest date interaction was detected for both pesticides in data detailing residue in above-soil surface plant biomass. Therefore, data were separated by DAT, and plant species are compared. All data presented regarding pesticide residue in plant biomass is calculated as a percent recovery relative to the mass applied at experiment initiation. At 14 DAT, 2,4-D residue in pickerelweed above-soil surface biomass (7.2% of applied) outranked arrow arum (0.9% of applied) and Virginia iris (1.2% of applied;
Table 5). Although data presentation inhibits statistical separation, 2,4-D residue in pickerelweed above-soil surface biomass declined 8-fold over the following 14 d (0.9% of applied at 28 DAT). 2,4-D residue in above-soil surface biomass did not differ between species at 28 DAT, ranging from 0.1 to 2.1% of the applied. Overall, 2,4-D residue declined in arrow arum and pickerelweed and increased in Virginia iris above-soil surface biomass from 14 to 28 DAT, with residue detection consistently occurring at the latest sample collection timing in the presented research. No differences occurred between plant species below-soil surface biomass 14 DAT (0.4 to 1.4% of applied) and 28 DAT (0.5 to 1.7% of applied); however, residue detection consistently occurred at the latest sample collection timing. Future research should extend sample collection periods to quantify 2,4-D persistence in plant biomass, which may affect management practices in pesticide mitigation areas. Interestingly, opposite residue trends in below-soil surface biomass within a species from 14 to 28 DAT occurred, with an increase in arrow arum and pickerelweed and decrease in Virginia iris. Further research is needed to elucidate pesticide uptake, translocation, and metabolism in the evaluated aquatic plant species.

Similar trends to 2,4-D occurred with azoxystrobin residue in plant above- and below-soil surface biomass (Table 6). Pickerelweed above-soil surface biomass contained 1.4% of azoxystrobin applied at 14 DAT, while decreased residue occurred in arrow arum (0.2% of applied) and Virginia iris (0.3% of applied). Similar residue in above-soil surface biomass occurred across plant species at 28 DAT, ranging from 0.5 to 1.0% of the applied. Lastly, no differences occurred between plant species below-soil surface biomass 14 DAT (0.1 to 0.4% of applied) and 28 DAT (0.1 to 0.5% of applied).
The research approach utilized limits a comprehensive understanding of pesticide persistence in the evaluated plants due to consistent detection at the last sample collection event. Additionally, residue analysis did not include metabolites, which would provide more insight on degradation and overall phytoremedial potential of the evaluated plants. Lastly, data do not differentiate between residue sorbed onto, or within the plant. Considering plant above- and below-soil surface biomass were inherently contacted with pesticide solution at experiment initiation, it is reasonable to expect a portion of the residue detected to follow differing bioremoval pathways. It can be confirmed from this research that 2,4-D and azoxystrobin persist in arrow arum, pickerelweed, and Virginia iris up to 1 month following exposure. Coupling this with previous research showing these compounds can release from vegetation suggests these compounds may cycle back into water if mechanically cut or following dormancy onset.\textsuperscript{13} Future research should investigate this phenomenon, as well as management practices and harvest timings to optimize pesticide removal from surface water bodies.

\textit{Plants Injury}

Visual estimates of plant injury suggest the evaluated species were differentially affected by 2,4-D and azoxystrobin exposure. Unacceptable injury, arbitrarily set at 50%, occurred following 2,4-D exposure on at least one plant species occurred at 7, 14, and 28 DAT (Table 7). At 7 DAT, arrow arum and pickerelweed injury ranged from 40 to 51%, while no injury occurred on Virginia iris. Similar trends occurred at 14 DAT, with 51 to 64% injury on arrow arum and pickerelweed, and reduced injury on Virginia iris (14%). Injury increased over time, with 78 to 100% across all plants observed by 28 DAT. Exposure
to azoxystrobin did not result in injury across from 7 to 28 DAT. Olette et al.\textsuperscript{17} conducted a conceptually similar experiment evaluating aquatic plant bioremoval of various pesticides, and reported flazasulfuron, an herbicide, resulted in greater plant toxicity than copper sulphate and dimethomorph, two fungicides. Overall, these data suggest aquatic plants are best suited to remove non-herbicide pesticides from surface water.

The evaluated 2,4-D concentration in water at initiation, which was selected for residue detection considerations, resulted in unacceptable injury at experiment completion. It should be noted that the 5 mg L\textsuperscript{-1} concentration exceeds the Clean Water Act, aquatic life benchmarks for vascular plants – acute exposure (0.013 mg L\textsuperscript{-1}).\textsuperscript{24} Furthermore, this rate exceeds the registered, maximum single application rate for aquatic weed control (4 mg L\textsuperscript{-1}).\textsuperscript{25} This highlights an area for improvement in future research quantifying herbicide bioremoval in phytoremedial settings. For example, research has shown pickerelweeds can effectively bioremove oryzalin and simazine, two herbicides widely used for grass weed control.\textsuperscript{26} Ultimately, future research should incorporate herbicides with differing modes of action and plants with differing physiology to better represent the range of compounds that may move off-target, and better understand their effect on diverse plant communities.

The initial azoxystrobin water concentration also exceeded the aquatic life benchmark for vascular plants – acute exposure (3.4 mg L\textsuperscript{-1}), although the magnitude in difference between initial concentration and benchmarks limits comparisons between 2,4-D and azoxystrobin.\textsuperscript{27} Injury was not observed throughout the experiment, which suggests the evaluated plants can tolerate azoxystrobin in many real-world exposure scenarios. Further
research is required to define 2,4-D azoxystrobin tolerance limits of across the evaluated plant species.

**Conclusions**

This research builds off previous efforts confirming aquatic plants can effectively remove pesticides from water. Azoxystrobin was comparably more readily removed from water than 2,4-D, which is thought to be in part due to herbicide activity from 2,4-D that altered plant growth, and resulted in reduced bioremoval. Virginia iris outranked arrow arum and pickerelweed in removing azoxystrobin residue in water at 7 DAT, suggesting it should be recommended for planting in pesticide mitigation areas neighboring turfgrass systems. However, this may be due to growth conditions specific to our experiment, and all species reduced > 75% azoxystrobin residue compared to non-planted containers by 14 DAT. Therefore, our results support the widely recommended practice of establishing diverse plant species in mitigation areas to optimize phytoremedial capacity and wildlife habitats. Although the evaluated 2,4-D water concentration at experiment initiation exceeded what would be anticipated in a pesticide mitigation area, species recommendations cannot be made from this research due to unacceptable injury at 28 DAT. Results suggest Virginia iris may effectively remove 2,4-D residue from water at lower initial concentrations; however, additional research is needed to confirm. Across pesticides and plant species, residue detection consistently occurred in above- and below-soil surface biomass at 28 DAT, which has not been previously documented in this setting and should be further investigated to identify potential mechanisms of pesticide cycling in mitigation areas. Future research
should also include plants of varying physiology evaluated individually, and in tandem to elucidate the potential for species diversity to optimize pesticide bioremoval from surface water.
References


Table 1. Physicochemical and toxicological properties of 2,4-D and azoxystrobin.

<table>
<thead>
<tr>
<th>Property</th>
<th>2,4-D Amine → Acid&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Azoxystrobin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Physicochemical</td>
<td>Toxicological</td>
</tr>
<tr>
<td></td>
<td>Herbicide</td>
<td></td>
</tr>
<tr>
<td>Pesticide class</td>
<td>33,900 to 796,000</td>
<td>Very high</td>
</tr>
<tr>
<td>Water solubility (mg L&lt;sup&gt;-1&lt;/sup&gt;; pH 7)</td>
<td>39</td>
<td>Non to slight</td>
</tr>
<tr>
<td>Hydrolysis half-life (d)</td>
<td>Stable</td>
<td>Non</td>
</tr>
<tr>
<td>Aqueous photolysis half-life (d)</td>
<td>19</td>
<td>Very high</td>
</tr>
</tbody>
</table>

<sup>a</sup> 2,4-D and azoxystrobin data obtained from United States Environmental Protection Agency documents.<sup>5,10</sup>

<sup>b</sup> Data presented for both due to rapid conversion of amine to acid in plants.<sup>5</sup>
Table 2. 2,4-D and azoxystrobin residue in water at 7, 14, and 28 days after treatment to pond water.\(^a\)

<table>
<thead>
<tr>
<th>Plant</th>
<th>2,4-D 7 DAT</th>
<th>2,4-D 14 DAT</th>
<th>2,4-D 28 DAT</th>
<th>Azoxystrobin 7 DAT</th>
<th>Azoxystrobin 14 DAT</th>
<th>Azoxystrobin 28 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrow Arum</td>
<td>5.0</td>
<td>3.7</td>
<td>1.8</td>
<td>5.5</td>
<td>2.7</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Pickerelweed</td>
<td>5.0</td>
<td>2.7</td>
<td>2.3</td>
<td>5.5</td>
<td>1.7</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Virginia iris</td>
<td>4.9</td>
<td>2.0</td>
<td>0.5</td>
<td>5.6</td>
<td>1.0</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Non-planted</td>
<td>5.1</td>
<td>4.3</td>
<td>4.1</td>
<td>5.6</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>NS</td>
<td>0.4</td>
<td>0.8</td>
<td>NS</td>
<td>1.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: DAT, days after treatment; ND, non-detection; NS, non-significant.
Table 3. Determination of azoxystrobin reduction in water from pickerelweed planted containers relative to non-planted containers 28 days after treatment to pond water.\(^a\)

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Water residue (mg L(^{-1}))</th>
<th>Water volume (L container(^{-1}))</th>
<th>Total azoxystrobin (mg container(^{-1}))</th>
<th>Water residue (mg L(^{-1}))</th>
<th>Water volume (L container(^{-1}))</th>
<th>Total azoxystrobin (mg container(^{-1}))</th>
<th>Azoxystrobin reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10</td>
<td>0.377</td>
<td>38</td>
<td>4.61</td>
<td>2.215</td>
<td>10,211</td>
<td>100(^a)</td>
</tr>
<tr>
<td>2</td>
<td>0.17</td>
<td>0.564</td>
<td>96</td>
<td>4.58</td>
<td>2.181</td>
<td>9,989</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>0.48</td>
<td>1.262</td>
<td>606</td>
<td>4.52</td>
<td>2.260</td>
<td>10,215</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>0.07</td>
<td>0.718</td>
<td>50</td>
<td>4.71</td>
<td>2.266</td>
<td>10,673</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\) % reduction = \{100 \times [1 - (mg ai within planted container / mg ai within non-planted container)]\}

% reduction = \{100 \times [1 - (38 / 10,211)]\} = 99.6 = 100%
Table 4. 2,4-D and azoxystrobin reduction in water relative to non-planted containers at 7, 14, and 28 days after treatment to pond water.\(^a\)

<table>
<thead>
<tr>
<th>Plant</th>
<th>2,4-D 7 DAT</th>
<th>2,4-D 14 DAT</th>
<th>2,4-D 28 DAT</th>
<th>Azoxystrobin 7 DAT</th>
<th>Azoxystrobin 14 DAT</th>
<th>Azoxystrobin 28 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrow Arum</td>
<td>15</td>
<td>77</td>
<td>74</td>
<td>21</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>Pickerelweed</td>
<td>21</td>
<td>78</td>
<td>77</td>
<td>37</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>Virginia iris</td>
<td>27</td>
<td>76</td>
<td>92</td>
<td>45</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>11</td>
<td>NS</td>
<td>3</td>
<td>6</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: DAT, days after treatment; NS, non-significant.
\(^b\) % reduction = \(\{100 \times [1 – (\text{mg ai within planted container} / \text{mg ai within non-planted container})]\}\)
Table 5. 2,4-D persistence in above- and below-soil surface biomass relative to the applied at experiment initiation.a

<table>
<thead>
<tr>
<th>Plant</th>
<th>14 DAT</th>
<th>28 DAT</th>
<th>14 DAT</th>
<th>28 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrow Arum</td>
<td>0.9</td>
<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Pickerelweed</td>
<td>7.2</td>
<td>0.9</td>
<td>0.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Virginia iris</td>
<td>1.2</td>
<td>2.1</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>LSD_{0.05}</td>
<td>2.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Abbreviation: DAT, days after treatment; NS, non-significant.
Table 6. Azoxystrobin persistence in above- and below-soil surface biomass relative to the applied at experiment initiation.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Plant</th>
<th>Above-soil surface</th>
<th>Below-soil surface</th>
<th>% of applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrow Arum</td>
<td>0.2 0.5</td>
<td>0.4 0.2</td>
<td></td>
</tr>
<tr>
<td>Pickerelweed</td>
<td>1.4 1.0</td>
<td>0.1 0.5</td>
<td></td>
</tr>
<tr>
<td>Virginia iris</td>
<td>0.3 0.7</td>
<td>0.1 0.1</td>
<td></td>
</tr>
<tr>
<td>LSD\textsubscript{0.05}</td>
<td>0.4 NS</td>
<td>NS NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviation: DAT, days after treatment; NS, non-significant.
<table>
<thead>
<tr>
<th>Plant</th>
<th>Arrow Arum 7 DAT</th>
<th>Arrow Arum 14 DAT</th>
<th>Arrow Arum 28 DAT</th>
<th>Pickerelweed 7 DAT</th>
<th>Pickerelweed 14 DAT</th>
<th>Pickerelweed 28 DAT</th>
<th>Virginia iris 7 DAT</th>
<th>Virginia iris 14 DAT</th>
<th>Virginia iris 28 DAT</th>
<th>LSD(_{0.05}) 7 DAT</th>
<th>LSD(_{0.05}) 14 DAT</th>
<th>LSD(_{0.05}) 28 DAT</th>
<th>% injury(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrow Arum</td>
<td>51</td>
<td>64</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>21</td>
<td>NS</td>
<td>16</td>
</tr>
<tr>
<td>Pickerelweed</td>
<td>40</td>
<td>51</td>
<td>98</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>16</td>
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<tr>
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<td>14</td>
<td>78</td>
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<td>0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>16</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: DAT, days after treatment; NS, non-significant. 
\(^b\) Injury visually estimated on a 0 to 100% scale, where 0 = no plant injury and 100 = complete plant death.