Factors governing zoysiagrass response to herbicides applied during spring green-up

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Abstract

Zoysiagrass (*Zoysia* spp.) is utilized as a warm-season turfgrass because of its density, visual quality, stress tolerance, and reduced input requirements. Turf managers often exploit winter dormancy in warm-season turfgrass to apply nonselective herbicides such as glyphosate and glufosinate to control winter annual weeds. Although this weed control strategy is common in bermudagrass (*Cynodon* spp.), it has been less adopted in zoysiagrass due to unexplainable turf injury. Many university extension publications recommend against applying nonselective herbicides to dormant zoysiagrass despite promotional language found in a few peer-reviewed publications and product labels. Previous researchers have used vague terminology such as “applied to dormant zoysiagrass” or “applied prior to zoysiagrass green-up” to describe herbicide application timings. These ambiguous terms have led to confusion since zoysiagrass typically has subcanopy green leaves and stems throughout the winter dormancy period. No research has sought to explain why some turfgrass managers are observing zoysiagrass injury when the literature only offers evidence that these herbicides do not injure dormant zoysiagrass. We sought to explore various herbicides, prevailing temperatures surrounding application, heat unit based application timings, and spray penetration into zoysiagrass canopies as possible contributors to zoysiagrass injury.

The results indicated that a wide range of herbicides may be safely used in dormant zoysiagrass. However, as zoysiagrass begins to produce more green leaves, herbicides such as metsulfuron, glyphosate, glufosinate, flumioxazin, and diquat become too injurious. Glufosinate was consistently more injurious regardless of application timing than glyphosate and other herbicides. When temperatures were 10 °C for 7 d following treatment, a delayed effect of glyphosate and glufosinate effect on digitally-assessed green cover loss was noted on zoysiagrass sprigs. In subsequent studies on turf plugs, a 14-d incubation period at 10 °C reduced glyphosate but not glufosinate effects on turf green color reduction. Glyphosate applied at 125, and 200 GDD_{5C} can safely be applied to zoysiagrass while glufosinate applied at the same timings caused inconsistent and often unacceptable zoysiagrass injury in field studies conducted at Blacksburg, VA, Starkville, MS, and Virginia Beach, VA. Zoysiagrass green leaf density was described as a function of accumulated heat units consistently across years and locations but variably by turf mowing height. Turf normalized difference vegetative index was primarily governed by green turf cover but reduced by herbicide treatments, especially when applied at greater than 200 GDD_{5C}. Substantial spray deposition occurred to subcanopy tissue regardless of nozzle type, pressure and height above the zoysiagrass canopy based on spectrophotometric assessment of a colorant admixture. However, increasing nozzle height above the turf canopy and avoiding air induction type nozzles significantly reduced the percentage of green tissue exposed at lower canopy levels. Absorption of radio-labeled glyphosate and glufosinate was up to four times greater when exposed to zoysiagrass stems compared to leaves. Glyphosate translocated more than glufosinate and both herbicides moved more readily from stem to leaf than from leaf to stem.

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General Audience Abstract

Zoysiagrass (*Zoysia* spp.) is utilized as a warm-season turfgrass because of its density, visual quality, stress tolerance, and reduced input requirements. Being that zoysiagrass is a warm-season turfgrass, it enters a dormancy period during the winter months. During this period, zoysiagrasses' active growth is halted, and leaves lose their green color and turn a golden-brown color. The winter dormancy period presents turfgrass managers with a unique opportunity to apply nonselective herbicides such as glyphosate and glufosinate to control a broad spectrum of winter annual weeds. Although this weed control strategy is common in bermudagrass (*Cynodon* spp.), it has been less adopted in zoysiagrass due to turfgrass managers observing unexplainable turfgrass injury. Many university extension publications recommend against applying nonselective herbicides to dormant zoysiagrass despite language found in peer-reviewed publications and product labels suggesting they could be safely applied. Previous researchers have used vague terminology such as "applied to dormant zoysiagrass" or "applied prior to zoysiagrass green-up" to describe herbicide application timings. These terms have led to confusion about when to make these applications since zoysiagrass typically has subcanopy green leaves and stems throughout the winter dormancy period. No research has sought to explain why some turfgrass managers observe zoysiagrass injury when the literature only offers evidence that these herbicides do not injure dormant zoysiagrass. Research projects were designed to explore various herbicides, temperatures surrounding herbicide applications, application timings, and spray penetration into zoysiagrass canopies as possible contributors to zoysiagrass injury.

The results indicated that a wide range of herbicides may be safely used in dormant and semidormant zoysiagrass. However, as zoysiagrass begins to produce more green leaves and stems, herbicides such as metsulfuron, glyphosate, glufosinate, flumioxazin, and diquat become too injurious and should be avoided. Across multiple research studies, glufosinate was consistently more injurious regardless of application timing than glyphosate and other herbicides. When temperatures were 10 °C for 7-d following treatment, it delayed zoysaigrass response to glyphosate and glufosinate. In a subsequent study, when temperatures were at 10 °C for a 14-d period, glyphosate and the nontreated reached 50% green cover at the same time, which suggests cold temperatures could mitigate glyphosate injury on zoysiagrass over a 14-d period. The 10 ° temperature only delayed glufosinate injury on zoysiagrass, and no safening was observed. The results also indicated that as temperatures increased, glyphosate and glufosinate rate in which injury was observed increased on the zoysiagrass.

Glyphosate applied at 125, and 200 GDD_{5C} can safely be applied to zoysiagrass while glufosinate applied at the same timings caused inconsistent and often unacceptable zoysiagrass injury in field studies conducted at Blacksburg, VA, Starkville, MS, and Virginia Beach, VA. Zoysiagrass injury increased when glyphosate and glufosinate were applied later into the spring when more green leaves were present regardless of location. Accumulated heat units and zoysiagrass green leaf density were closely related, indicating that accumulated heat units could be a useful tool for turfgrass managers to track zoysiagrass spring green-up. Substantial spray deposition was found on subcanopy zoysiagrass leaves and stems regardless of nozzle type, pressure, and height above the zoysiagrass canopy based on recovered colorant at the upper, middle and lower levels of the zoysiagrass canopy. However, avoiding air induction-type nozzles and raising spray height may slightly decrease penetration of spray droplets into a zoysiagrass subcanopy, but a large percentage of droplets still reached the middle and lower canopy layers in this research. Absorption of radio-labeled glyphosate and glufosinate was up to four times greater when applied directly to zoysiagrass stolen compared to leaves. Glyphosate translocated more than glufosinate, and both herbicides moved more readily from stem to leaf than from leaf to stem. These data suggest limiting the number of green zoysiagrass leaves at application would be an effective method to avoid injury zoysiagrass when applying nonselective herbicides.

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Table of Contents

List of Figures

List of Tables

Chapter 1. Introduction and literature review

Zoysiagrass (*Zoysia* ssp.) is a common C_4 warm-season grass that is native to the Southeast Asia region and commonly utilized on home lawns, athletic fields, and golf courses. Zoysia is a diverse genus, including *Zoysia japonica* Steud., *Zoysia matrella* (L) Merr, *Zoysia pacifica* (Goudswaard), and other species with genetic and morphological differences attributed to cultivar growth, quality, and performance (Chen et al., 2009; Patton and Reicher, 2007; Schwartz et al., 2010; Youngner, 1961). Zoysiagrass was introduced to the United States around 1900 and has been used on golf courses since the 1950s (Dunn and Diesburg, 2004). The cultivar ‘Meyer’ (*Zoysia japonica* Steud.), released in 1951, was the first zoysiagrass to be introduced to golf courses (Grau and Radko, 1951). The continued use of ‘Meyer’ by superintendents and researchers has made it the industry standard for zoysiagrass cultivars (Patton and Reicher, 2007).

Zoysia japonica Steud. and *Z. matrella* (L.) Merr. are currently the most widely used zoysiagrass species in the United States (Patton et al., 2004). *Zoysia japonica* Steud. species is commonly called Japanese, Chinese, or Korean Common lawngrass and was introduced into the United States as seed in 1930. ‘Meyer’, ‘El Toro’, ‘Zenith’, ‘Palisades’, ‘Empire’, and ‘Companion’ (Z. *japonica*) are coarser textured and have some of the best cold tolerance of the zoysiagrasses. The finer leaf textures of ‘Cavalier’, ‘Diamond’, ‘Zoro’, and ‘Zeon’ (Z. *matrella*) allow them to be mowed lower for golf course tees, and also have potential as a putting green grass. These cultivars require more maintenance and inputs compared to Z. *japonica* cultivars due to pest and thatch problems. Both Z. *japonica* and Z. *matrella* have good heat, drought, and wear tolerance but lack the rapid establishment rates of bermudagrass (*Cynodon dactylon* (L) Pers.) (Patton et al., 2017).

Zoysiagrass is primarily found in warm-arid and warm-humid climatic zones, but good winter hardiness allows for it to be used in the transition zone (Patton and Reicher, 2007). In recent years, zoysiagrass has become an increasingly popular turfgrass for home lawns and golf courses in the transition zone due to its slow growth rate, improved winter hardiness, wear tolerance, and low maintenance requirements (McCarty, 2011). Once established, zoysiagrass requires less nitrogen and mowing inputs than bermudagrass. Zoysiagrasses' primary limitations are shallow rooting, susceptibility to large patch (*Rhizoctonia solani*), and slow establishment and/or recuperative growth rates (McCarty, 2011; Patton et al., 2017). Specific limitations are dependent upon species and cultivar.

As of 2006, approximately 16,293 acres of zoysiagrass had been planted on golf courses in the U.S., with 81 % in the transition zone (Lyman et al., 2007). The transition zone is where cool-season and warm-season grasses encounter the limits of their southern and northern adaptations, respectively. Warm-season grass acreage in the transition zone is slowly increasing due to enhanced water use efficiency and capacity to tolerate heat stress compared to C_3 cool-season grasses (DiPaola and Beard, 1992).

The downside of growing warm-season grasses in the transition zone compared to cool-season grasses is a 4 to 6 month dormancy during winter months. However, zoysiagrass has typically been used more widely in the upper transitions zone compared to bermudagrass due to its superior low-temperature hardiness. Dormancy of turfgrass can be defined as a temporary suspension of visible growth of any plant structure (Lang, 1987). Dormancy can also be described as an escape from potentially harmful conditions by entering a dormant state. Warm-season turfgrass enters into winter dormancy as a natural response to adverse environmental conditions to protect its meristematic areas that will resume development when more favorable growing conditions return. Different environmental conditions determine the rates of growth and development of plants, with temperature being one of the most impactful and essential factors that can limit or enhance plant productivity. As winter approaches, day lengths shorten, and temperatures decrease below the optimum levels for growth and development of warm-season grasses. Low temperatures significantly reduce or halt chlorophyll synthesis, while existing chlorophyll is degraded by high light intensity (Beard, 1973; Hendry et al., 1987). Consequently, dormant warm-season turfgrass appears brown because there is no chlorophyll present in leaf tissue.

Starting in the fall, plants begin going through cold acclimation, which causes the growth of the plant to slow while simultaneously adjusting physiologically and metabolically to the changing environmental conditions. Photosynthesis rates are relatively high in the fall compared to respiration rates, which allows the plant to build carbohydrate reserves (Pompeiano et al., 2013). Fall accumulation of carbohydrates in tiller bases, crowns, stolons, and rhizomes is considered an important factor for cold tolerance and winter survival of many plant species, including turfgrasses (Dionne et al., 2001; Shahba et al., 2003). The discoloration is enhanced by stronger light intensity (Youngner, 1961). Dormancy continues to be induced when the average minimum air temperature for 15 consecutive days is below 15 °C, followed by termination of shoot growth at approximately 10 °C or below and soil temperature less than 16 °C (Baltensperger, 1962). Research has shown that zoysiagrass grown under 12/8 °C day/night temperatures went into dormancy in eight to ten days while zoysiagrass grown under 25/20 °C remained green regardless of photoperiod treatments (Wei et al, 2008). Carbohydrates stored in rhizomes and solons are primarily involved in dormancy recovery, regrowth, and recuperative capacity from cold stress (Goatley et al., 1999; Rimi et al., 2012).

Previous research has shown various environmental stress response differences between zoysiagrass species and cultivars, particularly in cultivars cold tolerance and spring and green-up rates. Pompeiano et al., 2013 found that Carbohydrates metabolism is one of the main factors involved in the development of cold hardiness identified in plants. Its also been found that zoysiagrass grown under shade had less rhizome mass and less nonstructural carbohydrate content than turf is grown in shaded conditions (Qian et al., 1998). The shading led to decreased cold tolerance by reducing the photosynthesis rate resulting in the limited synthesis of cryoprotectants and the accumulation of carbohydrates that are important for new growth in the spring (Steinke and Stier, 2003).

Many new cultivars of zoysiagrass that have since been released following ‘Meyer’ have finer leaf texture, and improved shade tolerance, establishment rate, divot recovery, and pest resistances compared to ‘Meyer’ (Karcher et al., 2005; Patton et al., 2007b; White and Engelke, 1990). However, ‘Meyer’ is still noted for its cold tolerance compared to newer cultivars. Patton and Reicher (2007) reported that Z. *japonica* suffered less winter injury and exhibited higher freeze tolerance compared to Z. *matrella* in laboratory freeze tests.

Throughout the transition zone, zoysiagrass typically enters dormancy in November and begins spring green-up in late March or early April (Dunn and Diesburg, 2004). During this time, it is susceptible to winter annual and perennial weed invasion. Weeds established in dormant turf reduce the aesthetic value, playability during winter and spring months and could reduce turf cover during spring green-up. In the transition zone many turf managers have adopted the strategy of winter applications of the nonselective herbicides, which is an effective method to control a broad spectrum of grassy and broadleaf weeds (Johnson, 1980; Velsor et al., 1989; Vargas Jr and Turgeon, 2003).

The majority of dormant herbicide research has involved bermudagrass because it is the most widely used turf in the Southeastern region of the U.S. (Johnson, 1976, 1977, 1980; Johnson and Ware, 1978; Johnson and Burns, 1985; Toler et al., 2007). Winter annual broadleaf weeds are most often controlled using two and three-way auxin herbicide mixtures. These mixtures have long been the standard but researchers have found certain hard to control weeds such as corn speedwell (*Veronica arvensis* L.), parsley-piert (*Aphanes arvensis* L.), and henbit (*Lamium amplexicaule* L.) often need repeat applications for full control (Derr and Serensits, 2016; Johnson, 1980). Research has indicated that glyphosate applied as a single treatment was more effective at controlling weeds compared to paraquat or a combination of 2, 4-D + mecoprop + dicamba (Johnson, 1976). Toler et al. (2007) also found glyphosate and glufosinate controlled annual bluegrass (*Poa annua* L.) 93 % when applied in February, but control was only 72 % with December application. Flessner et al. (2013) reported flumioxazin applied at 0.43 kg ai ha^{-1} controlled annual bluegrass at two tillers and less, but annual bluegrass with four and six tiller control was less than 50 %. Reed et al. (2015) reported tank mixing flumioxazin with flazasulfuron, glufosinate, glyphosate, and simazine were more effective than flumioxazin alone. Toler et al. (2007) reported sulfonylurea herbicides such as foramsulfuron, flazasulfuron, rimsulfuron, and trifloxysulfuron controlled annual bluegrass 87 % or greater. Johnson (1980) found that the spring growth of turf in the untreated plots was lower than in herbicide treatment plots because of competition from uncontrolled weeds. The reduction in control when herbicides were applied in early winter highlights the importance of timing herbicide applications in late winter to get the most effective weed control without regrowth of weeds.

Applying nonselective herbicides to dormant turf provides superintendents the ability to control hard to control weeds such as annual bluegrass; however, there is a risk of delaying spring green-up if the applications are applied while the turfgrass is breaking dormancy. Research has reported that glyphosate applied to semi-dormant or actively growing bermudagrass in the spring can delay spring green-up and cause injury (Johnson, 1977; Johnson, 1980; Johnson and Ware, 1978). However, results indicate herbicide applications that were made to dormant bermudagrass caused no injury or delay to green-up (Johnson, 1977; Johnson and Carrow, 1999; Johnson and Ware, 1978; Toler et al., 2007). It also has been reported that the 2,4-D, mecoprop, and dicamba combination reduces the quality and density of bermudagrass when applied to actively growing turf but not when applied to dormant or semi-dormant turf (Johnson and Burns, 1985). These results suggest that the timing of herbicide applications is crucial in avoiding delaying spring green-up, and it also suggests bermudagrass should be fully dormant when glyphosate applications are made (Johnson, 1984).

The growing popularity of zoysiagrass in the transition zone has increased the need to better understand and evaluate zoysiagrass tolerance to herbicides applied in the later winter and early spring. Much less research has been conducted to identify the timing of late winter and early spring herbicide applications for control of winter weeds and to determine the safety of zoysiagrass compared to bermudagrass. Velsor et al. (1989) found that paraquat and glyphosate applied on April 1 caused significant injury, but the same applications on March 1 caused no turf injury. Velsor et al. (1989) noted the turf visually appeared dormant, but close examination revealed 5-8 mm of green tissue at the base of the zoysiagrass stems on the April treatments. Carbon exchange rates measurements revealed that green leaf tissue was still photosynthetically active and able to absorb the herbicide. Rimi et al. (2012) conducted a similar study evaluating the effects of glyphosate applied at two different winter dates on weed control and spring green-up of a newer variety of zoysiagrass 'Zeon' (*Zoysia matrella*) zoysiagrass mown at 2.7 cm and 'Companion' (*Zoysia japonica*) zoysiagrass mown at 3.2 cm. Rimi et al. (2012) reported that glyphosate applied as a single treatment at 1.1kg ha^{-1} in February effectively controlled winter weeds in 'Zeon' zoysiagrass without injuring turf in the spring. However, late winter applied glyphosate delayed spring green-up of 'Companion' zoysiagrass from April 22 to the end of April. Results also indicated that visual green cover ratings were correlated with NDVI measurements (Rimi et al., 2012). Hoyle and Reeves (2017) applied glyphosate to dormant 'Meyer' zoysiagrass in early January and February and observed no zoysiagrass injury.

Recent research examined the weed control efficacy of glufosinate, applied at two rates, and glyphosate to determine potential turfgrass injury when applications were made within weeks or days before zoysiagrass broke dormancy (Xiong et al., 2013). The timings were based on historical records and scouting at each site, and dormancy was assessed visually. Treatment applications were made to a mature plot of two Z. *japonica* cultivars (‘Zenith’ mown at 2 cm and ‘Meyer’ mown at 5 cm) in the transition zone. In both years, neither glyphosate nor glufosinate influenced spring green-up when applied 2 or 3 weeks before green-up. However, glufosinate and glyphosate applications made at 2 to 3 days before dormancy reduced turf quality up to 40 %. The study suggests that glufosinate could be an alternative to glyphosate for weed management on dormant zoysiagrass turf when applied to dormant zoysiagrass at the proper timing (Xiong et al., 2013). The research that has been done suggests the effect of winter-applied herbicides on spring green-up could differ depending on zoysiagrass species/cultivars (Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013).

Although most of these studies examine weed control in what is described by the researchers as ‘dormant turf,’ the application timings and the level of dormancy at application obviously vary between studies. The dormant turf is usually visually rated, and some studies considered dormant, semi-dormant, or actively growing (Hoyle and Reeves, 2017; Rimi et al., 2013; Velsor et al., 1989; Xiong et al., 2013). Words such as semi-dormant turf are highly subjective and are not defined by how much green material is present in a given area of turf, which leads to a lack of understanding of what semi-dormant turf looks like. Velsor et al. (1989) noted turf could visually appear dormant, but close examination can reveal green tissue at the base of the stem. Tissue that is still green is photosynthetically active and able to absorb herbicide that might result in injury to the turf. The research that has been done has only examined a small portion of the available zoysiagrass varieties and has not given specific recommendations to ensure dormant applications of herbicides do not delay zoysiagrass green-up. Only a small portion of herbicides on the market have been evaluated for zoysiagrass tolerance during spring green-up. Herbicide applications in late winter are more desirable than earlier applications to improve overall weed control goals because of the limited residual activity for nonselective herbicides such as glyphosate and glufosinate. Therefore, it is vital to determine the proper timing of late winter and early spring herbicide applications to ensure zoysiagrass safety and effective weed control.

Air temperatures surrounding herbicide applications plays an essential role in herbicide efficacy (Derr and Serensits, 2016; Kudsk and Kristensen, 1992). Temperatures may influence the plants' cuticle development, morphology, and physiological process, altering herbicide translocation and absorption (Kudsk and Kristensen, 1992). Price (1983) noted as air temperatures increase, leaf cuticle waxes become less viscous, and the rate of herbicide absorption increases. In general, uptake and translocation of foliar-applied herbicides increase as temperatures increase (Anderson et al., 1993). McWhorter et al. (1980) found that ^{14}C-glyphosate absorption and translocation in johnsongrass (*Sorghum halepense* (L.) Pers.) increased as the air temperature increased from 24 °C to 35 °C. Jordan (1977) reported that the efficacy of glyphosate on bermudagrass improved at 32 °C compared to 22 °C. Pline et al. (1999) found similar results in that uptake of ^{14}C-glufosinate was significantly higher at 25 °C than at 15 °C. Low temperatures can also affect herbicide absorption and translocation. Anderson et al. (1993) noted cooler temperatures delayed the onset of glufosinate injury on green foxtail (*Seteria viridis* (L.) P. Beauv.). However, it did not remove the toxic effect but only increased the time it took to achieve control. Duke and Hunt (1977) observed that glyphosate translocation in quackgrass (*Agropyron repens* (L) Gould.) was reduced when exposed to low temperatures (7 °C). Research indicated that frost could influence the absorption and translocation of glyphosate (Devine and Bandeen, 1983).

Research has shown that herbicides applied during cold temperatures generally have less weed control efficacy than those applied during warm temperatures (Kells et al., 1984; Kieloch and Domaradzki, 2003). Sulfonylurea herbicides such as foramsulfuron, trifloxysulfuron, and flazasulfuron weed control efficacy can be negatively affected by cold temperatures in winter and early spring (Hutto et al., 2008; Lycan and Hart, 2006).

The ideal temperature to apply most postemergence herbicides is between 18 °C and 30 °C for the best weed control (Derr and Serensits, 2016; Kudsk and Kristensen, 1992). However, that is not always realistic in late winter and early spring. The environmental conditions vary during this time, leading to a wide range of turfgrass and weed growth stages in which herbicides could be applied. As the day length, air, and soil temperatures begin to rise in the spring zoysiagrass will begin to break dormancy and attempt to green-up. Periods of warmer temperatures often occur in later winter and early spring that favor some growth of zoysiagrass. Any new green vegetation that is present when nonselective herbicide applications are made could potentially lead to increased herbicide translocation to the meristematic areas and delay green-up or damage the turf. Research has yet to clearly outline application timings in the spring to ensure zoysiagrass safety.

Growing-degree-days (GDD) or heat unit accumulation are commonly used to measure or predict the effect of temperature on plant development throughout the growing season (Baskerville and Emin, 1969; McMaster and Wilhelm, 1997). The basic GDD equation used is $GDD = [T_{MAX} + T_{MIN})/2] - T_{BASE}$, where T_{MAX} is the daily maximum and T_{MIN} is the minimum air temperature and T_{BASE} is the lowest temperature at which the biological process of interest does not advance (McMaster and Wilhelm, 1997). Growing-degree-days are calculated as the average daily temperature above a base temperature, with each degree above the base temperature considered a degree day.

In turfgrass, GDD accumulation has many applications including spray scheduling programs for insect control, weed control, and plant growth regulator applications (Branham and Danneberger, 1989; Kreuser and Soldat, 2011; Reasor et al., 2018; Unruh et al., 1996). GDD accumulation can also predict annual bluegrass and Kentucky bluegrass (*Poa pratensis* L.) seedhead formation germination and smooth crabgrass (*Digitaria ischaemum* Schreb.) germination (Danneberger and Vargas, 1984; Fidanza et al., 1996; McCullough et al., 2017; Koski et al., 1988; Schlossberg et al., 2002). Brosnan et al. (2010) and Elmore et al. (2013) utilized GDD accumulation timings to determine application timings for control of bermudagrass and dallisgrass (*Paspalum dilatatum* Poir.). GDD accumulation also has been used to schedule applications of florasulam to suppress dandelion (*Taraxacum officinale* F.J. Wigg) bloom in the spring (Patton et al., 2018). Rimi et al. (2012) reported accumulated GDDs when glyphosate was applied to dormant zoysiagrass, but application timings were based on standard management practices used to control annual bluegrass, not GDD accumulation. Researchers have developed GDD models for zoysiagrass establishment, but none have examined the relationship between GDD accumulation and zoysiagrass green-up (Patton et al., 2004; Sladek et al., 2011). No previous research has examined GGD accumulation accuracy to predict herbicide application during spring green-up of zoysiagrass.

Application timings based on GDD accumulation can provide optimal estimates for when to initiate herbicide applications because the timing is made at approximately the same plant growth stage each year, rather than using an arbitrary calendar date. Although much research has been done examining the plant growth stage, no previous research has examined GGD based applications for herbicides applied during spring green-up. Being able to correlate GDD accumulation with green-up and the number of green leaves present at the application would give turf managers more information to ensure they do not injure their zoysiagrass.

A successful spray application in turfgrass settings not only depends on herbicide selection and the timing of the treatment, but it also is contingent on using the correct equipment and application techniques. Spray technology has improved over the past several years giving turfgrass managers numerous techniques to improve spray applications’ accuracy. Spray nozzle selection is a significant component in regulating the amount of spray applied to an area and the uniformity of spray coverage (Grisso et al., 2019). Nozzles form the spray pattern by breaking the liquid into droplets. The Volume Median Diameter (VMD) is often used to describe spray droplet size. The VMD refers to the median droplet size where half the volume of the spray is in droplet small and half of the volume is in droplets larger than the median. Droplet size classification system that ranges from extremely fine to ultra-coarse is based upon droplet volume measured in microns (µm). Spray nozzles generate droplets that range in diameter from 10 to greater than 1000 µm (Bouse et al., 1990).

The most common type of nozzles used in turfgrass settings is the conventional XR flat fan nozzle (Shepard et al., 2006). An XR flat fan nozzles form a narrow-tapered edge, flat fan spray pattern. The XR flat fan nozzle is often the choice of turf managers because of its excellent spray distribution (Shepard et al., 2006). Air induction nozzles (AI) are a more recent development in fan nozzle technology, where the air is sucked through a small opening by a venturi action causing aerated droplets. These nozzles have been shown to produce larger droplets with less drift potential than the standard flat fan nozzle (Piggott and Matthews, 1999).

The spray characteristics of nozzles are essential in herbicide applications because of their ultimate effect on the efficacy of the spray application. Spray droplet velocity and size can affect the spray deposition and drift of the droplets (Lake, 1977). Research has shown that, in general, larger droplet sizes correspond with high droplet velocities (Nuyttens et al., 2007). Nuyttens et al. (2007) noted that just because air induction nozzles produced large droplets, they could have less velocity than expected, mainly because the air in the droplet results in them being less heavy.

Foliar-applied herbicide applications are most effective when they result in maximum coverage and droplet distributions on the plant material (Ferguson et al., 2016b; Hislop, 1987). Research has shown that spray droplet characteristics can affect herbicide efficacy. Creech et al. (2016) reported glufosinate control of lambsquarter (*Chenopodium album* L.) increased using a fine spray ($D_{v0.5}$ 186 µm) classification compared to medium and coarse droplet sizes ($D_{v0.5}$ 470 to 516 µm). In contrast, glufosinate control of tomato (*Solanum lycopersicum*) and velvet leaf (*Abutilon theophrasti* Medik.) was greater using coarse droplets ($D_{v0.5}$ 470 to 628 µm) compared to fine droplets ($d_{v0.5}$ 186 µm) (Creech et al., 2016). Butts et al. (2018) examined the influence of spray droplet size and carrier volume on dicamba and glufosinate efficacy and discovered that as droplet size increased, weed control slightly decreased. Feng (2003) noted that glyphosate absorption increased as the droplet VMD increase from 180 µm (fine) to 490 µm (coarse). Brown et al. (2007) evaluated the efficacy of four corn (*Zea mays* L.) herbicides using two nozzles types (flat fan and air induction) and two spray pressures (280 kPa and 490 kPa). Results indicated flat fan nozzles provided better weed control than AI nozzles. There was an increase in weed control and increase in corn yield with the higher spray pressure with the AI nozzles. Etheridge et al. (1999) also noted that at 100 kPa the spray pattern was more slender compared to applications at 276 kPa when using venturi nozzles. Knoche (1994) pointed out that grasses with mostly vertical oriented leaf surfaces, herbicide performance increased more consistently as the droplets increased in size (Knoche, 1994). Ferguson et al. (2018) observed a reduction in paraquat efficacy using ultra coarse droplets producing nozzles and spray coverage was noted as the reason for poor efficacy.

Herbicide spray deposition can be defined as the number of spray droplets that reach a specific target (Knoche, 1994). Many factors can influence spray deposition, including nozzle type, carrier volume, height above canopy, and spray pressure (Fietsam et al., 2004; Knoche, 1994). In dormant zoysiagrass, where there are often green leaves present during winter dormancy, the goal is to make sure herbicide remains in the upper layer of the turfgrass canopy (Velsor et al., 1989). Spray penetration into the turfgrass canopy could lead to increased injury to the zoysiagrass. Research focusing on nozzles and turfgrass canopy penetration has primarily focused on disease control (Benelli, 2016; Fidanza et al., 2009; Kaminski and Fidanza, 2009; McDonald et al., 2006; Vincelli and Dixon, 2007). Research has primarily focused on spray application strategies to improve dollar spot (*Sclerotinia homoeocarpa* F.T. Bennet) control. These studies have indicated that spray applications that provide the greatest surface coverage by using various nozzle types and spray volumes tended to improve dollar spot control (Kaminski and Fidanza, 2009; McDonald et al., 2006; Vincelli and Dixon, 2007). Benelli (2016) provided the only research in turfgrass to examine how spray parameters could influence spray deposition on stem, sheath, and leaves in a zoysiagrass canopy. Results indicated that higher spray volumes increased the spray deposition on stems and sheaths (Benelli, 2016). Very little research has focused on spray application methods for weed control in turfgrass (Ferguson et al., 2016a; Neal et al., 1990). Neal et al. (1990) reported spray volume and nozzle type did not affect fenoxaprop weed control efficacy.

Research examining canopy penetration across a range of spray parameters and nozzles has been conducted in row crops. Bache (1985) discovered that regardless of droplet size, herbicide deposition decreased deeper into the crop's canopy and indicated that carrier volume had to increase to penetration dense crop canopy. Knoche (1994) suggested that increasing spray coverage by reducing droplet size and increasing carrier volume improved herbicide efficacy. Zhu et al. (2004) reported that AI nozzles produced the highest deposition through the peanut canopy compared to a conventional flat-fan nozzle. Derksen et al. (2008) observed fine spray droplets had reduced soybean canopy penetration compared to coarse droplets. Derksen et al. (2008) also noted smaller droplet nozzles produced slower-moving droplets that could have resulted in less spray droplets reaching the lower portion of the soybean canopy. Wolf and Daggupati (2009) indicated improved soybean canopy penetration from fine and medium spray droplets. Hanna et al. (2009) observed no difference in soybean canopy penetration when comparing nozzles and droplets.

The propensity of spray droplets to adhere to a plant surface could determine the quantity of herbicide available to be taken up by the plant. The vertical growth structure of grasses makes them more likely to retain small droplets than larger droplets (Knoche, 1994). Research examining corn plant ability to retain spray droplets indicated that medium and coarse droplets were retained on corn plants less than fine droplets; however, herbicide absorption and translocation increased with droplet size (Feng et al., 2003). When a spray droplet impacts the plant surface, it will either be deposited on the leaf or bounce, shatter, or roll-off (Creech et al., 2016). The droplets that do not adhere to the plant's surface can move through the canopy and may be deposited on plant material deeper into the canopy or reach the soil surface (Schou et al., 2011). High-speed cameras have observed spray droplets as small as 67 µm rebound off leaves (Reichard et al., 1986; Reichard, 1988). The larger droplets with a diameter greater than 400 µm may shatter upon impact (Grayson et al., 1991). It has been noted droplet retention increased as droplet size decreased.

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Chapter 2. Zoysiagrass and weed response to herbicides during spring green-up

Abstract

Winter annual weeds begin to germinate as zoysiagrass enters dormancy in the fall. Dense infestation of winter annual weeds can slow zoysiagrass green-up in the spring due to competition for sunlight, moisture and nutrients. A field study was conducted to evaluate broadleaf weed control and semidormant ‘Meyer’ zoysiagrass response to 17 herbicides during the springs of 2017, 2018, and 2020. A field study was conducted to investigate annual bluegrass control and dormant ‘Zeon’ zoysiagrass response to 18 herbicide treatments.

Glufosinate, glyphosate + simazine, and indaziflam + simazine were the only herbicides that controlled Persian speedwell over 90 % and significantly higher than auxin type herbicide combinations. The combination of simazine and glyphosate had better common dandelion and Persian speedwell than glyphosate applied alone. Glufosinate controlled common dandelion, hairy bittercress, and Persian speedwell more effectively than glyphosate. In the study examing broadleaf weed control, glyphosate and glufosinate controlled annual bluegrass equivalently. However, in the study targeting annual bluegrass control, glyphosate had greater annual bluegrass control than glufosiante. Herbicide treatments that contained simazine or foramsulfuron controlled annual bluegrass greater 90 %. The addition of flumioxazin to diquat, glufosinate, or glyphosate improved annual bluegrass control. The results indicate if ‘Meyer’ zoysiagrass has 5 % visual green turf cover metsulfuron, glyphosate, glufosinate and diquat should not be applied due to being too injurious. In both studies, glufosinate was more injurious to ‘Meyer’ and ‘Zeon’ zoysiagrass than glyphosate. These data suggest, most herbicides targeting annual bluegrass control can be safely applied to dormant ‘Zeon’ zoysiagrass, and a wide range of broadleaf herbicides can be applied safely to ‘Meyer’ zoysiagrass as its actively greening-up.

Nomenclature: ‘Meyer’ zoysiagrass, *Zoysia japonica* Stued.; ‘Zeon’ zoysiagrass, *Zoysia matrelle* L Mar.; annual bluegrass, *Poa annua* L.; Persian speedwell, *Veronica persica* Poir.; hairy bittercress, *Cardamine hirsute* L.; common dandelion, *Taraxacum officinale* F.H. Wigg.

Keywords: Zoysiagrass, turfgrass injury, dormant and semidormant zoysiagrass, annual winter weeds, annual bluegrass control, spring green-up.

Introduction

Zoysiagrass (*Zoysia* spp.) winter dormancy coincides with peak growth of winter annual weeds (Bingham et al., 1969; Johnson, 1977, 1980). If not controlled, these winter annual weeds can disrupt the aesthetic value of the zoysiagrass turfgrass and potentially slow zoysiagrass spring green-up by competing for space, water, and nutrient resources (Hall and Carey, 1992; Johnson, 1980). Both preemergence and postemergence herbicides are often utilized to control winter annual weeds in dormant turfgrass (Johnson and Carrow, 1999). Such herbicides have been extensively investigated in bermudagrass (*Cynodon dactylon* L.) (Johnson, 1976, 1984; Johnson and Ware, 1978; Toler et al., 2007), but few studies have reported their use in zoysiagrass. By investigating the literature related to herbicide use in bermudagrass and comparing herbicide label recommendations for use in both bermudagrass and zoysiagrass, one can discover a number of herbicides that are viable options for controlling most weed issues in dormant or semi-dormant zoysiagrass.

Annual bluegrass (*Poa annua* L.) has been reported as the most common and troublesome weed of turf and southern turfgrass systems that include dormant turfgrass management are no exception (Beard et al., 1978; Christians et al., 2011). Postemergence herbicides are commonly needed in the spring to control annual bluegrass on dormant turfgrass due to the weed's long germination period and ability to outlast the residual control period for some preemergence herbicides (Johnson and Burns, 1985; McElroy et al., 2004). Winter annual broadleaf weeds are most commonly controlled using herbicides that mimic growth hormones. Two and three-way mixtures of 2,4-D, dicamba, triclopyr, and MCPP have long been the standard for winter broadleaf weed control in bermudagrass (Johnson, 1975a). However, control of certain weeds such as corn speedwell (*Veronica arvensis* L.), parsley-piert (*Aphanes arvensis* L.), and henbit (*Lamium amplexicaule* L.) with these herbicides has been inconsistent when investigated in bermudagrass (Derr and Serensits, 2016; Johnson, 1980). A large selection of herbicides spanning hormone mimics, acetolactate synthase inhibitors, photosystem inhibitors, amino acid inhibitors, and cell wall biosynthesis inhibitors are routinely employed to control specific weed populations in dormant turfgrass (Brosnan et al., 2012b; Johnson, 1984; Johnson, 1975b; Reed et al., 2015; Toler et al., 2007).

Simazine is a photosystem II inhibiting herbicide that has preemergence and postemergence activity on annual bluegrass and broadleaf weeds (Johnson, 1982). Toler et al. (2007) found that simazine controlled annual bluegrass 86 % and 79 % in bermudagrass when applied in December and February, respectively. Simazine has also been shown to have excellent postemergence control of corn speedwell along with other broadleaf weeds (Johnson, 1982). Toler et al. (2007) reported that the ALS-inhibitors foramsulfuron, flazasulfuron, rimsulfuron, and trifloxysulfuron controlled annual bluegrass greater than 86 % in bermudagrass. Many turfgrass managers in the transition zone have adopted the strategy of winter applications of nonselective herbicides such as glyphosate, diquat, and glufosinate, which is an effective method to control a broad spectrum of grassy and broadleaf weeds in bermudagrass (Johnson, 1976, 1980; Johnson and Ware, 1978; Velsor et al., 1989). A few researchers have also reported the use of these nonselective herbicides in dormant or semidormant zoysiagrass (Hoyle and Reeves, 2017; Rimi et al., 2012; Velsor et al., 1989; Xiong et al. 2013). A single glyphosate application has been shown to control henbit, corn speedwell, and common chickweed (*Stellaria media* L.) more effectively than two and three-way growth regulator broadleaf products in dormant bermudagrass turf (Johnson, 1976). Xiong et al. (2013) reported glyphosate and glufosinate both effectively controlled annual bluegrass and mouse-ear chickweed (*Cerastium vulgatum* Hartm.) in dormant zoysiagrass.

Research has indicated that glyphosate and glufosinate applied to dormant zoysiagrass will not delay green-up (Hoyle and Reeves, 2017; Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013). However, if these chemicals are applied while turfgrass is exiting dormancy, green-up could be delayed (Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013). Tank mixing nonselective herbicides with residual products such as flumioxazin, oxadiazon, and indaziflam is often done by turfgrass managers in the spring to control existing weeds while also applying their residual product for summer annual weeds (Brecke et al., 2010; Brosnan et al., 2012b; Reed et al., 2015). Some of these combinations can be safely applied to actively growing turfgrass, but others must be applied to dormant turfgrass due to excessive injury caused when they are applied to actively growing turfgrass (Brecke et al. 2010; Johnson and Carrow, 1999; Reed et al. 2015). Few studies have reported zoysiagrass response to the majority of herbicides available for use in dormant turfgrass.

Since the majority of research involving winter annual weed control in turfgrass has been conducted primarily on bermudagrass, more information is needed to elucidate zoysiagrass response to herbicides used for this purpose. Herbicides such as flumioxazin, indaziflam, simazine, diquat, flazasulfuron, foramsulfuron, trifloxysulfuron, foramsulfuron, sulfentrazone, 2,4-D + dicamba + MCPP + carfentrazone, 2,4-D + 2,4-DP + MCPP + carfentrazone, MCPA + dicamba +

triclopyr, and 2,4-D + dicamba + fluroxypyr have not been examined for weed-control efficacy and zoysiagrass response during green-up. Therefore, studies were conducted to 1) evaluate numerous herbicides for winter broadleaf weed control and ‘Meyer’ zoysiagrass response and 2) evaluate several herbicide rates and mixtures for annual bluegrass control and ‘Zeon’ zoysiagrass response.

Materials and Methods

Broadleaf weed control and semidormant ‘Meyer’ zoysiagrass response to herbicides

Five field trials were conducted at the Virginia Tech Turfgrass Research Center (TRC) in Blacksburg, VA (37.214472, -80.411476), to evaluate broadleaf weed control and zoysiagrass response to 17 herbicides during the springs of 2017, 2018, and 2020. On March 27, 2017, at 250 GDD_{5C}, a field trial was initiated on a mixed stand of broadleaf weeds with no desirable turfgrass present, and an additional year was established on weed-free ‘Meyer’ zoysiagrass. On March 17, 2018 at 168 GDD_{5C}, these two trials were repeated at adjacent locations. Because the tolerance year from 2017 was confounded by winterkill and large patch issues, this study was repeated on March 18, 2020 at 156 GDD_{5C} on a uniform area of ‘Meyer’ zoysiagrass. The following weeds were evaluated : common chickweed (*Stellaria media* L.), Persian speedwell (*Veronica persica* Poir), common dandelion (*Taraxacum officinale* F.H. Wigg.), annual bluegrass and hairy bittercress (*Cardamine hirsute* L). On average, weedy plots contained approximately 10 % common chickweed, 40 % Persian speedwell, 7 % common dandelion, and 10 % hairy bittercress at trial initiation. All weeds were mature at application timing. Soil for all trials was a Groseclose urban land complex (clayey, mixed, mesic, Typic Hapludalf) with pH of 5.4 ± 0.2 and organic matter of 3.5 ± 0.3 % depending on year. The weed control trials were mown with a rotary mower at 3.8 cm twice a week during active growth with clippings being returned. No irrigation, fertilizer, or pesticides were applied to the trial years during the studies. The zoysiagrass tolerance trials were mown with a reel mower at 1.3 cm twice a week during active growth with clipping being returned. On average, plots had between 5 ± 3 % visual green zoysiagrass cover and 114 ± 30 green leaves dm^{-2} at treatment application with the majority of these leaves located below the upper canopy. Zoysiagrass green leaf counts were collected by counting all green leaves present within a 10-cm-by-10-cm, randomly chosen location in each plot, counting all leaves within the canopy that were at least half green and extrapolating to dm^{-2}. The treatment timing for these studies was intended to replicate a spring herbicide application to a semidormant (but actively greening-up) zoysiagrass when broadleaf weeds are most commonly targeted by turf managers using selective herbicides

The experimental design was a randomized complete block with four replicates, and plots were 1.7 m^2. All trials were applied with a CO_2-powered backpack sprayer calibrated to apply 280 L/ha. Herbicide treatments and rates can be found in Table 1. Weed control, zoysiagrass green-up, zoysiagrass injury, and normalized difference vegetative index (NDVI) data were collected at 0, 7, 14, 21, 28, 42, 56, and 70 days after treatment (DAT). Weed control was assessed visually on a 0 to 100 % scale, where 0 indicates that plots had equivalent green weed vegetation compared to the nontreated and 100 % indicates all green vegetation of the target weed was eliminated. Zoysiagrass green cover was assessed as a visually estimated percentage of plot area. Zoysiagrass injury was assessed similar to weed control on a 0 to 100 % scale based on visually estimated loss of green vegetation. Injury that 30 % and greater was consider unacceptable turfgrass injury.

Measurements of NDVI were collected using a Holland Scientific Crop Circle ACS 210 active crop sensor (Holland Scientific Inc., Lincoln, NE) affixed 43 cm above the turf that collected 50 ± 5 readings per plot that represented a 0.5 x 1.6 m area of turf canopy in the center of each plot.

Data Analysis

Zoysiagrass green cover, NDVI, and turf injury measurements over time were converted to the area under progress curve (AUPC) using

$$\partial = \sum_{i=1}^{ni-1} \left(\frac{(y_i + y_{(i-1)})}{2} (t_{(i+1)} - t_{(i)} \right), \qquad \text{Eq 1}$$

where ∂ is the AUPC, *i* is the ordered sampling date, ni is the number of sampling dates, *y* is turf weed cover, green-up NDVI or turf injury measurements at a given date, and *t* is the time in days. The AUPC was then converted to the average d^{-1} by dividing by the number of days spanned by the assessment period. Campbell and Madden (1990, p. 192–193) applied this equation to disease epidemiology, and Askew et al. (2013) and Brewer et al. (2017) utilized it for weediness over time in a turfgrass comparison study. The AUPC is useful in situations where long durations are assessed by repeated measures. This technique offers a better comparison between treatments when the measured response’s severity and duration are variable such as herbicide-induced injury that persist for several weeks and when the timing of maximum injury may vary between disparate herbicide modes of action. The AUPC d^{-1} data derived from control of five weed species, zoysiagrass green cover, turf canopy NDVI, and zoysiagrass injury were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect year, treatment, rep, and year x treatment effects. The four included years were considered a single random variable and mean square error associated with treatment effects were tested with the mean square associated with year x treatment (McIntosh, 1983). A separate analysis for common chickweed and hairy bittercress control was conducted for 28 DAT to capture maximum control. Similarly, dandelion, Persian speedwell, and annual bluegrass control analysis was done separately for 56 DAT. Data were discussed separately by year if significant year x treatment interaction was detected ($P < 0.05$). Otherwise, data were pooled over year. Appropriate interactions or main effects were subjected to Fisher's Protected LSD test at $\alpha = 0.05$ to compare means.

Poa annua control and dormant 'Zeon' zoysiagrass response to herbicides

Field trials were established in 2016, 2017, 2018, and 2020 to investigate annual bluegrass control and 'Zeon' zoysiagrass response to 18 herbicide treatments. The first three studies were conducted at the Westlake Golf Course (Westlake) in Hardy, VA (37.130761, -79.718261), and the 2020 study was conducted at the Glade Road Research Facility (GRRF) in Blacksburg, VA (37.233250, -80.435983). Herbicides were initially applied on Mar 1, 2016, Feb 17, 2017, Mar 16, 2018, and Feb 26, 2020, at 30, 100, 164, and 87 GDD_{5C}, respectively. Zoysiagrass was mown at 1.3 cm using a reel mower three times per week during active growth in all years with clipping returned. Soil at Westlake was a Clifford fine sandy loam (fine, kaolinitic, mesic Typic Kanhopludults) 6.3 pH, and 3.2 % organic matter. At the GRRF, soil was a Duffield silt loam (fine-loamy, mixed, active, mesic, Ultic Hapludalfs)-Ernest silt loam (fine-loamy, mixed, superactive, mesic Aquic Fragiudults) complex, with a pH of 6.8 and 3.5 % organic matter. All years had a natural annual bluegrass infestation at 30 to 50 % coverage of 10- to 20-tiller plants at Westlake and 3 to 5 % coverage of 5- to 10-tiller plants at the GRRF. Zoysiagrass had less than 20 subcanopy green or partially green leaves dm^{-2} and no visible green cover at application for all years. No pesticides, irrigation, or fertility were applied to the trial years while the experiment was in progress.

Studies were arranged as randomized complete block designs with three replications. Plots at Westlake were 1.8 by 3.6 m, and plots at the GRRF were 1.2 by 1.8 m. Herbicide treatments and rates can be found in Table 3. Treatments were applied with a CO_2 powered boom sprayer equipped with TTI 11004 nozzles (TeeJet Technologies, Springfield, IL) calibrated to deliver 280 L ha^{-1}. Annual bluegrass cover and control, zoysiagrass green-up and injury, and NDVI data were collected at 0, 7, 14, 21, 28, 42, 56, 70, and 84 DAT using methods previously described in the broadleaf control study. In 2018, zoysiagrass response data was confounded by winterkill and disease and were omitted from the analysis.

Data Analysis

Annual bluegrass cover, annual bluegrass control, zoysiagrass green cover, NDVI, and turf injury measurements over time were converted to the area under progress curve (AUPC) d^{-1} using the same formula and parameters as mentioned previously. Maximum observed turfgrass injury was also recorded as the highest injury data recorded on any assessment date. A separate analysis of annual bluegrass cover and control was also conducted for the final rating date at 84 DAT. These data were subjected to ANOVA with sums of squares partitioned to reflect year, treatment, replication, and year x treatment effects. Mean square tests and mean separations were conducted as previously described.

Results and Discussion

Broadleaf weed control and semidormant ‘Meyer’ zoysiagrass response to herbicides

The interaction of year x herbicide was not significant for common chickweed (P = 0.6428) and hairy bittercress (P = 0.1020) control at 28 DAT or for Persian speedwell (P = 0.2356) and annual bluegrass (P = 0.1010) control at 56 DAT (Table 1). The main effect of herbicide was significant for all four of these weeds (P < 0.0001); therefore, data were pooled over years. The interaction of year x herbicide treatment was significant for common dandelion control at 56 DAT (P = < 0.0001); therefore, data were presented separately for the two years where this weed was evaluated.

Diquat, florasulam, foramsulfuron, glufosinate, glyphosate, simazine, and metsulfuron applied alone or in mixtures with other herbicides controlled common chickweed at least 80 % 28 DAT (Table 1). Auxin-type herbicides in various combinations and sulfentrazone did not control common chickweed greater than 74 %. Slightly reduced control by auxin-type herbicides and sulfentrazone have been noted by other researchers when herbicides are applied during cold temperatures to blooming plants (Derr and Serensits 2016; Raudenbush and Keeley, 2014). Glyphosate controlled hairy bittercress 83 % at 28 DAT and less than diquat or glufosinate but equivalent to most other herbicides (Table 1). Other research in container-grown crops has indicated that mecoprop + 2,4-D + dicamba control hairy bittercress variably between 50 to 100 % (Altland et al., 2000) but information is lacking on controlling hairy bittercress in turfgrass settings. Hairy bittercress and common chickweed evaluation period was shortened compared to the other weeds evaluated each year because of sensitivity to warm temperatures and rapidly completing their floral development.

The common dandelion year interaction at 56 DAT was likely caused by variable responses to glyphosate + simazine, indaziflam, and sulfentrazone (Table 1). All other treatments responded similarly between years. Glufosinate controlled common dandelion 96 to 99 % and orders of magnitude better than glyphosate or diquat. Glyphosate controlled common dandelion significantly better when mixed with simazine. Glufosinate was similar to auxin-mimic products, florasulam containing treatments, and metsulfuron-containing treatments in that they were all equivalent to the best common dandelion control observed in the study. Variable and limited control of certain broadleaf weeds by glyphosate has been reported (Jordan et al., 1997; Koger et al., 2007; Koger et al., 2004; Shaw and Arnold, 2002). The results from this study are also similar to other research findings in that three and four-way herbicide combinations, including auxin-type products, are effective options to control common dandelion (Raudenbush and Keeley, 2014).

Glufosinate, glyphosate + simazine, and indaziflam + simazine were the only herbicides that controlled Persian speedwell over 90 % at 56 DAT (Table 1). Auxin-type herbicides did not control Persian speedwell greater than 38 %. Poor control of Persian speedwell and other speedwell species by auxin-type herbicides has been reported (Johnson, 1976). Brosnan et al.

(2012a) observed similar results in that the combination of metsulfuron+sulfentrazone had higher broadleaf weed control than either alone (Brosnan et al., 2012a). However, these results did not translate to the other broadleaf weeds evaluated in this study.

Glyphosate and glufosinate controlled annual bluegrass equivalently at 77 to 80 % (Table 1). It should be noted, however, that glufosinate was applied at the maximum-allowable rate for dormant turfgrass while glyphosate was applied at approximately 30 % of the maximum allowable rate. Other researchers have reported similar annual bluegrass control between glyphosate and glufosinate in zoysiagrass turf (Toler et al., 2007; Xiong et al., 2013). Treatments that contained simazine or foramsulfuron controlled annual bluegrass over 90 % and better than other treatments. Previous research has indicated that foramsulfuron and simazine are effective annual bluegrass control options (Johnson, 1982; Toler et al., 2007). However, over-reliance on simazine, glyphosate, and sulfonylurea herbicides such as foramsulfuron for annual bluegrass control has led an increase in reported annual bluegrass resistance to these herbicides (Breeden et al., 2017; Brosnan et al., 2020; Hutto et al., 2004).

The interaction of year x herbicide was significant for zoysiagrass green cover, NDVI, and injury AUPC d^{-1} ($P < 0.0001$); therefore, data are presented separately by year (Table 2). Varying weather conditions during the spring could have caused the interaction between year and treatments for green cover AUPC d^{-1}. The temperatures following treatment in 2018 were much warmer, leading to more rapid green turf cover, while 2020 temperatures were much cooler, resulting in slower green-up. Specific treatments that may have caused the interaction include any combination containing glufosinate, glyphosate, or metsulfuron (Table 2). Increased herbicidal injury during the warmer 2018 conditions likely caused the reduced cover d^{-1} in 2018 compared to 2020 for treatments containing these herbicides. Other treatments were reasonably consistent between years with respect to green cover AUPC d^{-1}. Untreated zoysiagrass maintained an average green cover d^{-1} of 70 to 82 % (Table 2). It should be noted that treatments were initiated at approximately 5 % green turf cover and reached near 100 % cover in nontreated plots by 56 DAT (data not shown). Thus, the AUPC d^{-1} in nontreated plots is lower than one may expect due to reduced green cover as turf was initially breaking dormancy during the first half of the evaluation period. Dinalli et al. (2015) noted that metsulfuron caused severe phytotoxicity to 'Emerald' zoysiagrass. Dernoeden (1994) concluded that metsulfuron was too injurious to 'Meyer' zoysiagrass. Glufosinate reduced zoysiagrass green cover d^{-1} more than all other treatments (Table 2). Cover reduction or "delayed green-up" has been reported when both glyphosate and glufosinate have been applied to semidormant zoysiagrass turf (Xiong et al., 2013).

The interaction of year x treatment for NDVI AUPC d^{-1} was caused by several treatments having lower NDVI d^{-1} from the colder conditions of 2020 compared to 2018 (Table 2). As with green cover trends, glufosinate, glyphosate, and metsulfuron were among the treatments that varied between years, but several other treatments were also variable. Normalized difference vegetative idex trends may have been confounded by dead or dying weeds, variable temperatures and frost-associated zoysiagrass injury, or other unknown factors between years. The NDVI d^{-1} data did support observations of reduced cover d^{-1} by glufosinate in that glufosinate caused lower NDVI values compared to all other treatments at both years, just as it did regarding green cover.

Upon examining injury AUPC d^{-1}, it is evident that higher injury levels in the warmer 2018 spring season compared to the cooler 2020 spring season caused the year x treatment interaction for injury as well as cover and possibly NDVI. In 2018, glyphosate, diquat, glufosinate, metsulfuron + sulfentrazone, and glyphosate + simazine were the only treatments that had an injury AUPC d^{-1} above an acceptable injury threshold of 30 % (Table 2). In 2018, glyphosate averaged an injury AUPC d^{-1} of 32 %, while glufosinate had an injury AUPC d^{-1} of 83 % (Table 2). In 2020, glufosinate was the only treatment to have an injury AUPC d^{-1} above 30 % (Table 2). In order to average 30 % injury d^{-1} during the entire evaluation period, these herbicides had to be extremely injurious to the turf or cause moderate levels of injury that persisted for most of the 70-d evaluation period. Thus, any treatment with an injury AUPC d^{-1} of greater than 20 is likely too injurious to use on semidormant zoysiagrass turf. Glyphosate and glufosinate have injured zoysiagrass if applied when small percentages of green cover were apparent at treatment time (Rimi et al., 2012; Xiong et al., 2013). However, previous research has not observed glufosinate being more injurious than glyphosate (Xiong et al., 2013). The weather conditions following the initiation of 2020 were not as warm and did not favor green-up or herbicidal activity. Late frosts appeared to halt slow green-up, possibly masking some of the herbicide injury or partially safening the turfgrass due to reduced herbicidal activity. Weather conditions following 2018 treatments were more favorable to green-up and herbicidal activity, allowing for maximum contrast between rapidly growing green turf and stunted or discolored turf.

Poa annua control and dormant 'Zeon' zoysiagrass response to herbicides

The year x herbicide interaction was insignificant for annual bluegrass cover AUPC d^{-1} ($P = 0.1065$), cover at 84 DAT ($P = 0.1168$), annual bluegrass control AUPC d^{-1} ($P = 0.0601$), annual bluegrass control at 84 DAT ($P = 0.0874$). The herbicide main effect was significant for all of these responses ($P < 0.0001$); Therefore, data were pooled over years (Table 3). Annual bluegrass control by both glyphosate and glufosinate were rate dependent. Glufosinate and diquat controlled annual bluegrass less than glyphosate based on both AUPC d^{-1} and observed values at 84 DAT with the exception of glyphosate versus glufosinate each at higher rates for AUPC d^{-1} only (Table 3). The comparison of glyphosate and glufosinate at high rates being similar for control d^{-1} but different at 84 DAT suggest that initial annual bluegrass control was equivalent for the two, as seen in other work (Toler et al., 2007; Xiong et al., 2013), but annual bluegrass recovered more over time following glufosinate treatment. The addition of flumioxazin to diquat, glufosinate, or glyphosate improved annual bluegrass control d^{-1} and final control at 84 DAT but such improvement did not occur when oxadiazon was added to glufosinate or glyphosate. Reed et al. (2015) observed similar results in that flumioxazin tank-mixed with glyphosate was more effective than flumioxazin alone. Our results contrast with reports that combining contact-type mode of actions with glyphosate could decrease weed control (Wehtje et al., 2008; Wehtje et al., 2010).

Foramsulfuron controlled annual bluegrass 92 % at 84 DAT, which was equivalent to glyphosate at 520 g ae ha^{-1} and glyphosate at 390 g ae ha^{-1} + flumioxazin and better than all other treatments. Among sulfonylurea herbicides, annual bluegrass control was greatest to least in the following order: foramsulfuron > trifloxysulfuron > metsulfuron + rimsulfuron > flazasulfuron (Table 3). Foramsulfuron and trifloxysulfuron have been superior-performing sulfonylurea treatments in previous research (Harrell et al., 2005; Toler et al., 2007). Flazasulfuron has also been shown to inconsistently control annual bluegrass. Harrell et al. (2005) reported 49 % annual bluegrass control in 2002 and 92 % control in 2003. Toler et al. (2007) reported flazasulfuron controlled annual bluegrass 97 %. Performance of flazasulfuron may have been reduced due to the maturity of annual bluegrass when applications were made.

Annual bluegrass cover AUPC d^{-1} in nontreated plots was slightly higher numerically than final cover observations at 84 DAT. This occurred because the initially observed cover was near maxima, and maxima was reached between 28 and 42 DAT then declined slightly as temperatures warmed entering the summer season (data not shown). These cover data reflect that annual bluegrass infestations were severe but indicative of winter weed issues on dormant turf. Annual bluegrass infestations were so extensive that we expanded our experimental plot sizes to 3.6 m long at Westlake to improve the assessment of zoysiagrass response to herbicides. The best-performing treatments based on annual bluegrass control, which included glyphosate at 520 g ae ha^{-1}, glyphosate at 390 g ae ha^{-1} + flumioxazin, and foramsulfuron, still had 4.0 to 8.6 % annual bluegrass cover at 84 DAT (Table 3). Regardless of the rate, glyphosate and glufosinate annual bluegrass cover AUPC d^{-1} was significantly less than diquat (Table 3). Only diquat at both rates and oxadiazon alone did not alter annual bluegrass cover AUPC d^{-1} or final cover at 84 DAT when compared to the nontreated (Table 3). These data suggest diquat applied in the spring does not reduce annual bluegrass cover and are in agreement with other research (Toler et al.

2007). Overall, trends in annual bluegrass cover and AUPC d^{-1} data were negatively related to that of annual bluegrass control.

The interaction between year x herbicide was not significant for injury maxima ($P = 0.0641$) and green cover ($P = 0.3810$) (Table 4). The herbicide main effect was significant for injury maxima ($P = 0.0003$) and green cover ($P = 0.0263$); therefore, data were pooled over year. The interaction between year x herbicide was significant for injury ($P < 0.0001$); and NDVI ($P = 0.0039$) AUPC d^{-1}; therefore, data are presented separately (Table 4). With the exception of metsulfuron + rimsulfuron, no herbicide injured 'Zeon' zoysiagrass more than 25 % at any assessment date based on injury maxima analysis (Table 4). These data agree with other reports regarding minimal injury response when glyphosate and glufosinate were applied to dormant turf (Rimi et al., 2012; Xiong et al., 2013). Glufosinate applied at 1680 g ai ha^{-1}, flumioxazin, flumioxazin + glyphosate, and flumioxazin + glufosinate; however, all had an injury maxima of 22 to 25 % (Table 4). These near-threshold levels of injury are concerning since data from Table 2 and other reports (Rimi et al., 2012; Xiong et al., 2013; Velsor et al., 1989) have indicated that herbicide injury may substantially increase as zoysiagrass green cover begins to increase in the season. Thus, these herbicides may be safely applied during dormancy but must be scrutinized if any zoysiagrass green cover is detected at the canopy surface. Metsulfuron + rimsulfuron had the highest injury maxima compared to all other treatments at 39 % and would not be recommended for use in dormant zoysiagrass (Table 4). Injury maxima of glufosinate at both rates was significantly higher than glyphosate at both rates (Table 4).

The interaction between year x herbicide treatment for injury AUPC d^{-1} can primarily be attributed to variable zoysiagrass response to metsulfuron + rimsulfuron between years. In 2016, spikes in initial injury were followed by rapid recovery compared to more persistent injury levels in 2017 and 2020 and a substantial change of several orders of magnitude in injury AUPC d^{-1} data between these years. Glufosinate at the high rate caused significantly more injury d^{-1} than glyphosate at the high rate in two of three years. Injury d^{-1} generally agree with injury maxima data. Variable recovery speed following initial injury may explain slight changes in injury d^{-1} between years.

Interactions of year x herbicide treatment for NDVI AUPC d^{-1} could be attributed to varying levels of green cover, weed density, and herbicidal activity between years (Table 4). The 2017 data were collected at Westlake, where annual bluegrass populations comprised over half of the turf, while the 2020 data were collected at the GRRF where annual bluegrass populations were only 5 % of the turf canopy. In general, herbicides like glyphosate at 390 g ae ha^{-1} that controlled annual bluegrass well but did not injure zoysiagrass, had lower NDVI d^{-1} in 2017 compared to other treatments but comparable to the best NDVI d^{-1} in 2020. These differences are largely driven by herbicidal effects on the large annual bluegrass populations at Westlake in 2017. In 2020, NDVI d^{-1} better supports trends in herbicidal injury in that the more injurious herbicide treatments glufosinate at 1680 g ai ha^{-1} and metsulfuron + rimsulfuron had among the lowest NDVI d^{-1} (Table 4).

The trends in zoysiagrass green cover AUPC d^{-1} generally suggest that most treatments were minimally injurious to dormant zoysiagrass (Table 4). Only three treatments had green turf cover d^{-1} that was lower than the nontreated. These included metsulfuron + rimsulfuron, flumioxazin, and flumioxazin + glufosinate, all among the more injurious treatments.

Results of these studies suggest that a wide range of herbicides may be used in dormant ‘Zeon’ and ‘Meyer’ zoysiagrass. Metsulfuron + rimsulfuron, however, is too injurious to zoysiagrass when applied soon before spring green-up. High rates of glufosinate and flumioxazin do not injure dormant zoysiagrass unacceptably but warrant some concern regarding injury potential. When ‘Meyer’ zoysiagrass with 5 % green turf cover is treated with herbicides, metsulfuron, glyphosate, glufosinate, and diquat are too injurious at the rates tested in these studies. Glufosinate shows a tendency to be more injurious to zoysiagrass than glyphosate or diquat, regardless of dormancy level. This finding contrasts with the only paper that has compared glufosinate and glyphosate in similar situations and found them equivalent for zoysiagrass response (Xiong et al., 2013). Broadleaf weed control by the herbicide treatments tested is weed-species dependent, but a wide range of products can safely be used for that purpose. Annual bluegrass control is best with products that contain glyphosate, high rates of glufosinate, foramsulfuron, and simazine.

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Table 1. Influence of herbicide treatment on common chickweed and hairy bittercress control at 28 d after treatment and annual bluegrass, dandelion, and Persian speedwell control at 56 d after treatment of various herbicides applied in March of 2017, 2018, and/or 2020 when zoysiagrass green turf cover was 5 ± 3 %, turf had 114 ± 30 green zoysiagrass leaves dm^{-2} distributed throughout the canopy, and growing-degree-days at base 5°C were 216 ± 46. Dandelion control was dependent on year, but all other weed responses are pooled over at least two site years.

		Common	Hairy	Dandelion		Persian	Annual
Treatment	Rate (g/ha)	chickweed	bittercress	2017	2018	speedwell	bluegrass
		Control %					
2,4-D + dicamba + fluroxypyr	1680 + 210 + 210	73	78	95	97	31	0.6
MCPA + dicamba + triclopyr	1470 + 147 + 147	68	80	94	96	28	0.6
2,4-D + dicamba + MCPP + carfentrazone	857 + 269 + 78.4 + 28.0	74	90	91	99	38	0.0
2,4-D + 2,4-DP + MCPP + carfentrazone	286 + 168 + 28.0 + 22.4	59	89	91	91	28	0.0
Diquat	560	94	95	10	14	44	30
Florasulam	14.7	82	68	94	96	14	0.6
Foramsulfuron	28.2	83	70	85	93	62	96
Foramsulfuron + florasulam	28.2 + 14.7	87	73	91	95	79	93
Foram. + halosulfuron + thiencarbazone	28.2 + 43.1 + 13.9	83	75	83	95	83	95
Glufosinate	1680	99	99	96	99	92	77
Glyphosate	520	98	83	46	40	82	80
Glyphosate + simazine	520 + 2240	99	79	69	90	99	99
Indaziflam	32.6	56	77	53	15	64	17
Indaziflam + simazine	32.6 + 2240	99	86	58	43	99	97
Metsulfuron	21.0	91	84	86	99	59	14
Metsulfuron + sulfentrazone	42.0 + 420	96	85	89	99	78	21
Sulfentrazone	280	62	55	81	35	21	4.8
LSD (0.05)		6.7	10	8.8	11	7.8	7.6

Table 2. Influence of herbicide treatment on average daily zoysiagrass green cover, normalized difference vegetation index, and visually estimated injury based on area under the progress curve following seven assessments over a 70-d period after treatment when various herbicides were applied in March of 2018 and 2020 when zoysiagrass green turf cover was 5 ± 3% and turf had 82 ± 11 green zoysiagrass leaves dm^{-2} distributed throughout the canopy, and growing-degree-days at base 5 °C were 162 ± 6.

		Green cover		NDVI		Injury	
Treatment	Rate (g/ha)	2018	2020	2018	2020	2018	2020
		———— AUPC d^{-1} ————					
Nontreated	--	70	82	0.687	0.629	--	--
2,4-D + dicamba + fluroxypyr	1680 + 210 + 210	69	81	0.679	0.612	0.0	0.0
MCPA + dicamba + triclopyr	1470 + 147 + 147	70	77	0.600	0.554	1.1	4.0
2,4-D + dicamba + MCPP + carfentrazone	857 + 269 + 78.4 + 28.0	70	78	0.685	0.557	0.4	2.9
2,4-D + 2,4-DP + MCPP + carfentrazone	286 + 168 + 28.0 + 22.4	67	82	0.670	0.635	0.0	0.0
Diquat	560	63	72	0.600	0.562	39	19
Florasulam	14.7	70	80	0.688	0.593	0.0	0.0
Foramsulfuron	28.2	66	78	0.673	0.591	9.0	0.0
Foramsulfuron + florasulam	28.2 + 14.7	64	79	0.663	0.592	11	6.0
Foram. + halosulfuron + thiencarbazone	28.2 + 43.1 + 13.9	64	76	0.678	0.591	9.4	1.8
Glufosinate	1680	19	45	0.386	0.507	83	57
Glyphosate	520	50	78	0.610	0.589	32	1.9
Glyphosate + simazine	520 + 2240	44	77	0.573	0.574	35	4.1
Indaziflam	32.6	69	79	0.682	0.604	1.3	0.1
Indaziflam + simazine	32.6 + 2240	72	78	0.704	0.582	0.0	0.4
Metsulfuron	21.0	53	72	0.609	0.591	25	21
Metsulfuron + sulfentrazone	42.0 + 420	46	71	0.595	0.550	32	21
Sulfentrazone	280	67	80	0.685	0.599	1.9	0.0
LSD (0.05)		2.5	3.6	0.028	0.043	3.8	4.1

Table 3. Influence of herbicide treatment on final and average-daily annual bluegrass cover and control. Average daily values are based on area under the progress curve following nine assessments over an 84-d period after treatment when various herbicides were applied on late February to early March of 2016, 2017, 2018, and 2020 when zoysiagrass had no green turf cover and less than 20 subcanopy green or partially green leaves dm^{-2} and growing-degree-days at base 5°C were 97± 67. All responses are pooled over four site years[a].

		Annual bluegrass control		Annual bluegrass cover	
Treatment	Rate (g/ha)	AUPC d^{-1}	84 DAT	AUPC d^{-1}	84 DAT
Nontreated	--	--	--	59	50
Diquat	280	13	6.3	52	49
Diquat	560	17	5.4	51	46
Diquat + flumioxazin	280 + 428	41	45	36	24
Diquat + oxadiazon	280 + 3383	28	18	43	38
Glufosinate	840	53	38	28	32
Glufosinate	1680	77	65	14	19
Glufosinate + flumioxazin	840 + 428	76	75	13	11
Glufosinate + oxadiazon	840 + 3383	56	40	26	31
Glyphosate	390	72	75	13	14
Glyphosate	520	80	88	10	6.6
Glyphosate + flumioxazin	390 + 428	78	88	14	8.6
Glyphosate + oxadiazon	390 + 3383	74	71	12	14
Flazasulfuron	52.5	46	51	33	27
Flumioxazin	428	45	54	25	14
Foramsulfuron	28.9	65	92	18	4.0
Metsulfuron + Rimsulfuron	21.0 + 17.5	53	62	28	23
Oxadiazon	3383	5.4	3.4	55	47
Trifloxysulfuron	18.3	62	80	23	16
LSD (0.05)	--	4.2	7.9	9.4	9.9

[a] For three years between 2016 and 2018, studies were conducted on separate 1.3-cm 'Zeon' zoysiagrass fairways with natural infestations of annual bluegrass comprising 30 to 50 % turf coverage of 10- to 20-tiller plants at Westlake Golf Course in Hardy, VA. In 2020, a study was conducted on a 1.3-cm research fairway of 'Zeon' zoysiagrass with a natural infestation of annual bluegrass comprising 3 to 5 % coverage of 5- to 10-tiller plants at the Glade Road Research Facility at Virginia Tech in Blacksburg, VA.

Table 4. Influence of herbicide treatment on percentage maximum injury and average daily values of percentage injury, normalized difference vegetation index, and percentage green cover based on area under the progress curve following nine assessments over an 84-d period after treatment when various herbicides were applied on late February to early March of 2016, 2017, and 2020 when zoysiagrass had no green turf cover and less than 20 subcanopy green or partially green leaves dm^{-2} and growing degree days at base 5 °C were 97 ± 67. Percentage injury maxima and green cover AUPC d^{-1} are pooled over three site years. Injury and NDVI were separated by three and two years, respectively, due to significant year x treatment interaction. Zoysiagrass response data from an additional trial in 2018 was confounded by winter kill and disease and was omitted.

		Injury	Injury			NDVI		Green
Treatment	Rate	maxima	2016	2017	2020	2017	2020	cover
	(g ha^{-1})	%	AUPC d^{-1}					
Nontreated	--	--	--	--	--	0.426	0.611	44
Diquat	280	1.0	0.0	0.8	0.0	0.412	0.618	44
Diquat	560	3.6	1.0	1.4	0.4	0.425	0.580	42
Diquat + flumioxazin	280 + 428	10	2.0	5.5	3.3	0.392	0.543	41
Diquat + oxadiazon	280 + 3383	6.7	4.3	0.8	0.0	0.416	0.574	39
Glufosinate	840	13	7.3	2.5	0.0	0.421	0.617	41
Glufosinate	1680	25	7.2	7.8	7.9	0.392	0.501	41
Glufosinate + flumioxazin	840 + 428	22	7.0	7.4	11.2	0.376	0.539	38
Glufosinate + oxadiazon	840 + 3383	9.0	0.0	4.7	2.6	0.397	0.590	42
Glyphosate	390	3.8	1.0	0.7	0.0	0.398	0.612	44
Glyphosate	520	8.2	2.3	4.5	0.0	0.388	0.589	45
Glyphosate + flumioxazin	390 + 428	25	11	7.5	9.4	0.399	0.554	42
Glyphosate + oxadiazon	390 + 3383	7.6	1.7	2.9	2.0	0.384	0.608	43
Flazasulfuron	52.5	9.6	2.5	2.8	0.6	0.419	0.621	43
Flumioxazin	428	22	10	4.9	8.6	0.408	0.557	39
Foramsulfuron	28.9	4.4	2.0	0.7	0.3	0.418	0.599	44
Oxadiazon	3383	2.8	1.7	0.0	0.2	0.437	0.605	42
Metsulfuron + Rimsulfuron	21.0 + 17.5	39	4.0	11	28	0.427	0.551	37
Trifloxysulfuron	18.3	9.4	5.4	3.1	0.2	0.438	0.592	42
LSD (0.05)		6.7	4.4	4.0	4.6	0.037	0.036	4.0

Chapter 3. Temperature and heat units influence zoysiagrass response to glyphosate and glufosinate

Abstract

Turfgrass managers in the transition zone have adopted the strategy of late winter applications of both nonselective and selective herbicides on bermudagrass but are hesitant to make these applications to zoysiagrass due to fear that it may not be "completely dormant." When these applications are being applied, temperatures are often sporadic and unpredictable, which could lead to variable zoysiagrass response to herbicides. Research has shown that air temperatures can affect weed control and crop safety from herbicides. However, no research has examined the impact of temperature during or after non-selective herbicide applications on zoysiagrass response during spring green-up. A growth chamber study was conducted to evaluate zoysiagrass sprigs response to glyphosate and glufosinate applications as influenced by three different temperature regimes during and after treatment. An additional study was conducted on intact zoysiagrass plugs to evaluate the impact of an extended period (14 days) of the three temperature regimes during and after nonselective herbicides application on zoysiagrass response. A field research study was conducted at two unique sites each spring in 2016 and 2017 in Blacksburg, VA, to evaluate the influence of variable heat unit accumulation on zoysiagrass response to 7 herbicides.

Data indicate that zoysiagrass sprigs treated with glufosinate required less time to reach a 50 % green cover reduction than glyphosate, regardless of rate. Zoysiagrass sprigs incubated for 7 days at 10 °C slowed the rate of green cover reduction for both herbicides, but upon moving to the 27 °C temperature after 7 days, green cover was quickly reduced. When zoysiagrass plugs were incubated at 10 °C for 14 days after treatment, the nontreated and glyphosate-treated plugs both reached 50 % green cover in 22 days compared to 70 days for plugs treated with glufosinate. These data suggest that the cold temperature over a 14-d duration may have safened zoysiagrass plugs to glyphosate treatment but not to glufosinate treatment. These data also suggest that glyphosate activity on ‘Meyer’ zoysiagrass is considerably more temperature-dependent than glufosinate. Data from both the sprig and plug studies indicate that glufosinate may be more injurious to ‘Meyer’ zoysiagrass than glyphosate based on speed of activity. Foramsulfuron and oxadiazon applied at 200 and 300 GDD_{5C} can be safely used on zoysiagrass. Glufosinate, flumioxazin, diquat, metsulfuron + rimsulfuron would be deemed too injurious to use at application timings of ~ 200 GDD_{5C} or later. These studies indicate that as the number of green leaves within a zoysiagrass canopy increased, the injury observed from the herbicides tested increased.

Nomenclature: Bermudagrass, *Cynodon dactylon* L.; Zoysiagrass, *Zoysia* spp.; ‘Meyer’ Zoysiagrass, *Zoysia japonica* Steud.

Keywords: Zoysiagrass, turfgrass injury, temperatures, glyphosate, glufosinate, heat units, growing degree days.

Introduction

Turfgrass managers often apply nonselective herbicides such as glyphosate and glufosinate to control winter annual weeds in dormant zoysiagrass (*Zoysia* spp.) (Johnson, 1980; Toler et al., 2007; Xiong et al., 2013). Cold temperatures and shorter day lengths are needed to induce this dormancy period (Baltensperger, 1962; Beard, 1972; Hendry et al., 1987). Nonselective herbicide use during zoysiagrass dormancy is more common in the climatic transition zone of the United States or areas that represent the northern extent of the zoysiagrass growing region (Lyman et al., 2007; Patton et al., 2017). More southern areas seldom receive winter temperatures cold enough for zoysiagrass to lose all of its green foliage in the upper canopy and much confusion exists as to the safety of herbicide use on zoysiagrass during winter. In fact, the concept of "dormancy" in zoysiagrass is poorly understood and inadequately described in the scientific literature (Patton et al., 2017). Zoysiagrass dormancy is associated with "straw or golden brown color" of the canopy foliage (Patton et al., 2017), but plants can vary in green color retention when progressing into the winter season (Pompeiano et al., 2014) and often exhibit subcanopy green leaves or stems in late winter when nonselective herbicides are typically applied (Velsor et al., 1989).

Zoysiagrass injury from glyphosate or glufosinate is of great concern to turfgrass managers as these herbicides can substantially delay development of green turfgrass in spring and reduce zoysiagrass quality (Rimi et al., 2012; Xiong et al., 2013). Only one study has reported how zoysiagrass responds to glyphosate and glufosinate during late winter (Xiong et al., 2013), and the two herbicides were said to reduce zoysiagrass quality equivalently when applied later in the spring season to partially green turfgrass. Of the four studies that have evaluated either glyphosate or glufosinate on zoysiagrass during winter (Hoyle and Reeves, 2017; Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013), all indicate that these herbicides are safe to apply on "dormant zoysiagrass” or “prior to green-up”, but only one paper (Velsor et al., 1989) made any attempt to characterize these terms. Velsor et al. (1989) described green tissue at the base of zoysiagrass stems that was 3 mm long and produced measurable carbon exchange rates but claimed the canopy was otherwise brown when herbicides were applied to “dormant turfgrass.” Despite all four reports indicating safety to zoysiagrass when using either glyphosate or glufosinate during dormancy, one researcher suggested that differences in temperature minima between sites in Italy may have influenced zoysiagrass green cover accumulation following three levels of glyphosate treatment to dormant *Zoysia matrella* (L) Merr. (Rimi et al., 2012).

It has been documented that different species of zoysiagrass are strongly responsive to temperature (Patton and Reicher, 2007). In fact, variable response to cold temperatures is said to be the determining factor for the geographic distribution of zoysiagrass species, with *Z. japonica* being more adapted to colder regions than *Z. matrella*, such as the climatic transition zone of the United States (Patton et al., 2017). The few reported cases of nonselective herbicides injuring zoysiagrass have all been related to applying the herbicides later in the season during warmer temperatures (Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013). Weather patterns in the transition zone are irregular when turf managers are planning to apply herbicides to control winter annual weeds with variation in temperature occurring within a few days. The ideal temperature to apply most postemergence herbicides to control winter annual weeds in the spring and later winter is between 18 and 30 °C to achieve the best weed control (Derr and Serensits, 2016; Kudsk and Kristensen, 1992).

Temperature can directly affect herbicide efficacy by influencing plant growth and development (Kudsk and Kristensen, 1992). Changes in temperature can cause physiological changes in the plant resulting in the rate at which herbicides are absorbed and translocated (Muzik and Mauldin, 1964; Price, 1983; Varanasi et al., 2016). Research indicates that herbicides applied during warm conditions are often more effective than applications made during cold temperatures (Derr and Serensits, 2016; Kudsk and Kristensen, 1992). Jordan (1977) indicated that increasing temperatures from 22 C to 32 °C significantly increased glyphosate activity on bermudagrass (*Cynodon dactylon* L.). McWhorter et al. (1980) reported similar temperature responses when glyphosate absorption and translocation increased in johnsongrass (*Sorghum halepense* (L.) Pers.) as temperature increased from 24 C to 35 °C. Similar positive correlations between glufosinate absorption and temperature have also been reported (Pline et al., 1999b). It has been shown that temperatures below 10 °C can reduce herbicide adsorption and slow activity (McWhorter et al., 1980). Duke and Hunt (1977) reported that glyphosate translocation was reduced when plants were exposed to 7 °C. Low temperatures have also delayed injury to glufosinate-treated green foxtail (*Setaria viridis* (L.) P. Beauv.) and barley (*Hordeum vulgare* L.) (Anderson et al., 1993; Mersey et al., 1990).

Our own observations and those of Velsor et al. (1989) indicate that some level of green tissue will be present when most zoysiagrass is treated in winter. We hypothesized that green zoysiagrass shoots or zoysiagrass canopies with small amounts of green leaf tissue will respond to glyphosate and glufosinate differently at different temperatures during and after treatment. We further hypothesized that zoysiagrass green cover and the number of green leaves in zoysiagrass canopies will increase with increasing heat units resulting in greater zoysiagrass injury from glyphosate, glufosinate, and other selected herbicides. Research regarding the effects of temperatures or heat units on zoysiagrass response to herbicides during spring green-up has not been previously reported. Therefore, the objectives of this research were to 1) determine zoysiagrass response to glyphosate and glufosinate applications as influenced by three different temperature regimes during and after treatment, 2) determine the impact of extended periods (greater than 7 days) of three temperatures during or after nonselective herbicide applications on zoysiagrass response during spring green-up, and 3) determine the influence of variable heat unit accumulation on in-field zoysiagrass growth parameters and injury response to herbicides.

Materials and Methods

Herbicide and temperature effects on zoysiagrass sprigs

A growth-chamber experiment was conducted six times at the Virginia Tech Glade Road Research Facility in Blacksburg, VA (37.233250, -80.435983) from March to May 2018. The study was arranged in a randomized complete block, split-plot design with three temperatures as main plots and factorial arrangement of subplots with two herbicides applied at three rates as subplots, six temporal blocks with one replication each, and five subsample sprigs per experimental unit. 'Meyer' (*Zoysia japonica*) zoysiagrass plugs were collected from a field site mown regularly at 6.35 cm. Once removed from the field, intact plugs were acclimated in a greenhouse at 27 ± 6 °C for 48 hours with approximately 420 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) and irrigated every 24 hours. Following the incubation period, plugs were dissected to select newly-emerging green shoots (sprigs) that were approximately 5 cm long and had basal nodes. Five sprigs were dipped into a 0.59 mg ai L^{-1} solution of fluxapyroxad + pyraclostrobin (Lexicon Intrinsic fungicide, BASF Corporation, Research Triangle Park, NC) to prevent disease and placed in 8.9-cm diameter petri-dishes on blotter paper discs (76# Heavy Weight Seed Germination Paper, Anchor Paper Co., St. Paul, MN) (Amaradasa et al., 2014). Petri-dishes were then placed into three separate growth chambers set to constant 10, 18, and 27 °C with constant lighting that delivered 330 μmol m^{-2} s^{-1} of PAR. Temperatures throughout the experiment in the growth chambers and greenhouse were monitored using external data loggers equipped with temperature sensors (Onset Corp. using HOBO U12 4-channel outdoor external data loggers and TMCE 20-HD air/water/soil temperature sensors, Bourne, MA) programmed to record temperatures at 15 min intervals. Sprigs were allowed to acclimate at respective temperatures for 12 hours and then removed from petri dishes, over-sprayed with glyphosate (Roundup Pro Concentrate herbicide, Bayer Environmental Sciences, Research Triangle Park, NC) at 130, 260, or 520 g ae ha^{-1} or glufosinate (Finale herbicide, Bayer Environmental Sciences, Research Triangle Park, NC) at 420, 840, or 1680 g ai ha^{-1}, and returned to growth chambers. A nontreated was included for each temperature regime.

Herbicide treatments were applied with a CO_2-pressurized spray chamber calibrated at 280 L ha^{-1} using an XR 8003E even flat spray tip (Teejett Technologies, Springfield, IL). For the duration of the experiment, water was applied directly to the blotter paper disc to moisten and all petri dishes were covered with glass lids to preserve moisture. A liquid nutrient solution (20-20-20) was applied at 7.3 kg ha^{-1} to each petri dish three days after herbicide treatments were applied. Seven days after treatments were applied, petri-dishes were moved to the greenhouse and incubated for an additional fourteen days. The greenhouse temperature was maintained at 27 °C ± 6 °C, and supplemental light provided 420 μmol m^{-2} s^{-1} PAR for 13-hour each day.

The green cover was assessed by collecting ariel digital images of each petri dish at 0, 2, 4, 7, 10, 14, 18, and 21 days after treatment (DAT). The digital images were analyzed using Sigma Scan 5 to detect green pixels (Hue 42-100 and Saturation 30-100) (Karcher and Richardson, 2005). At trial completion 21 days after treatment, the treated sprigs were oven dried for 48 hours and weighed. Detected green pixels of treated sprigs were converted to percent reduction relative to nontreated sprigs for each temperature by replicate combination. Percent reduction was subjected to sigmoidal nonlinear regression (Eq. 1) to explain the relationship of time after treatment.

$$C = ae^{be^{kT}} \tag{1}$$

where C is percentage green cover, a is the upper asymptote for final green cover, b and k are constants, e is the base of natural logarithm, and T is time in days after treatment. To determine the number of days required to reduce green cover by 50 % (GCR_{50}). A unique GCR_{50} value was determined for each experimental unit (Eq. 2)

$$GCR_{50} = -(ln(-((ln(50/a))/b))/k) \tag{2}$$

where GCR_{50} is the time in d to reach 50 % reduction in green cover, a, b, and k are estimated parameters from equation 1, and ln is the natural logarithm. The GCR_{50} values and final dry biomass of sprigs were subjected to ANOVA in SAS 9.2 (SAS Institute, Cary, NC). Sums of squares were separated to account for temperature, replication, replication x temperature, herbicide, and temperature x herbicide as appropriate for the split-plot design (McIntosh, 1983). Mean squares associated with effects or interactions containing herbicide were tested residual error. Mean squares of the main plot temperature were tested by rep x temperature (McIntosh, 1983). Appropriate means were separated with Fisher's Protected LSD ($P \leq 0.05$).

Herbicide and temperature effects on plugs of zoysiagrass turf

A separate experiment was initiated in the spring of 2019 and repeated in the spring of 2020 to evaluate extended periods of temperatures surrounding glyphosate and glufosinate applications. The experiment was arranged as a randomized complete block study that included four replications in time in 2019 and an additional four replications completed in 2020. Treatments were arranged in a split-plot design of three temperature main plots and three herbicide subplots. 'Meyer' zoysiagrass plugs that were were removed from the same location as the first growth chamber experiment. Once the zoysiagrass plugs were removed from the field, the intact plugs were placed into 14-cm diameter pots backed filled with native soil. After the plugs were potted, they were over-sprayed with fluxapyroxad + pyraclostrobin (Lexicon Intrinsic fungicide, BASF Corporation, Research Triangle Park, NC) at 644 g ai ha^{-1} to prevent disease. They were then placed in the greenhouse for 48 hours, to begin greening-up. When zoysiagrass plugs had approximately 10 % visual green cover in the upper canopy, they were moved into three growth chambers set to constant 10, 18, and 27 °C with constant light at 330 μmol m^{-2} s^{-1} of PAR. After 12 hours, plugs were removed from the growth chambers and were over-sprayed with glyphosate at 520 g ae ha^{-1} or glufosinate at 1680 g ai ha^{-1} and returned to chambers. A nontreated was included for each temperature regime. Herbicide treatments were applied as previously discussed. No irrigation was applied until three days after herbicide treatment to ensure herbicides had adequate time to be absorbed. Irrigation was applied over the top of plugs as needed while in growth chambers. Fourteen days after treatments were applied, the plugs were moved to the greenhouse and incubated for an additional 42 days. Greenhouse conditions were maintained as previously discussed.

Zoysiagrass green cover was assessed via aerial images and digital image analysis as previously discussed. Spectral reflectance data were collected using a handheld, hyperspectral field radiometer (PSR-1100F, Spectral Evolution, MA) fitted with a plant probe measuring a spot size of 2.5 cm directly on the turfgrass canopy. The radiometer was routinely calibrated for reflectance between replications using white BaSO4 calibration panels. Two subsamples were collected per plug and averaged. The reflectance data was utilized to calculate the normalized difference vegetative index (NDVI) [(R_{760}-R_{670})/(R_{760}+R_{670})] (Carrow et al., 2010; Rouse et al., 1974). The NDVI data of turfgrass canopies has been closely related to turfgrass quality and green cover Carrow et al., 2010). Data were collected at 0, 2, 4, 7, 10, 14, 21, 28, 42, and 56 DAT.

Unlike the sprig study where plant material started mostly green and green cover either expanded due to growth or was reduced by herbicides over time, plugs in this study were mostly brown at initiation, and green cover increased over time. Thus, we did not convert to a percentage reduction in green cover but rather used equations 1 and 2 to evaluate green turf cover accumulation over time based on detected green pixels compared to total pixels available in the pot area. To detect the number of days required to reach green turf cover of 50 % (GTC_{50}), equation 2 was used with the Y variable replaced with GTC_{50} instead of GCR_{50} (Eq. 2).

NDVI data over time were converted to the area under progress curve (AUPC) (Eq. 3) using

$$\partial = \sum_{i=1}^{ni-1} \left(\frac{(y_i + y_{(i-1)})}{2} (t_{(i+1)} - t_{(i)} \right), \qquad [3]$$

where ∂ is the AUPC, *i* is the ordered sampling date, ni is the number of sampling dates, *y* is NDVI at a given date, and *t* is the time in days. The AUPC was then converted to the average d^{-1} by dividing by the number of days spanned by the assessment period. Campbell and Madden (Campbell and Madden, 1990, p. 192–193) applied this equation to disease epidemiology, and Askew et al. (2013) and Brewer et al. (2017) utilized it for weediness over time in a turfgrass comparison study. The AUPC is useful in situations where long durations are assessed by repeated measures. NDVI AUPC d^{-1} data were separated and analyzed as NDVI in-chamber AUPC d^{-1} for plugs and NDVI post chamber AUPC d^{-1} for plugs to examine the possible differences in turf recovery once removed from the growth chambers. The GTC_{50} and NDVI AUPC d^{-1} data were subjected to ANOVA with sums of squares separated to account for year, temperature, replication, replication x temperature (year), herbicide, temperature x herbicide, year x herbicide, and year x temperature x herbicide as appropriate for the split-plot design (McIntosh, 1983). Mean squares associated with effects or interactions containing herbicide were tested by their interaction with year. Mean squares of the main plot temperature were tested by replication x temperature with year nested (McIntosh, 1983). Appropriate means were separated with Fisher's Protected LSD ($P \leq 0.05$).

Heat unit effect on zoysiagrass turf response to herbicides

A field research study was conducted at two unique sites each spring in 2016 and 2017 at the Virginia Tech Turfgrass Research Center in Blacksburg, VA (37.214472, -80.411476). Each year, one of the sites contained a mixed stand of 'Companion' (*Zoysia japonica* L.) and 'Zenith' (*Zoysia japonica* L.) zoysiagrass mown with a rotary mower at 5 cm, and the second site consisted of 'Meyer' (*Zoysia Japonica* L.) zoysiagrass mown with a reel mower at 1.5 cm. The soil at both trial sites was a Groseclose urban land complex (clayey, mixed, mesic, Typic Hapludalf) with pH ranging from 6.3 to 6.5 and organic matter ranging from 3.2 to 4.8 %. Both sites were mown twice per week during active growth with clippings returned to the canopy. In both years, fertility, pesticides, or irrigation were withheld from the sites while the experiment was in progress.

The experiment was arranged as a randomized complete block design with a two (application timings) by seven (herbicides) factorial treatment arrangement with three replications. Plots were 1.8 by 1.8 m. Seven herbicide treatments were evaluated and are listed with associated rates in table 2. Treatments were applied with a CO_2-powered boom sprayer equipped with four

Turbo Teejet Induction 11004 spray tips (TeeJet Technologies, Springfield, IL) calibrated to deliver 280 L ha^{-1}. Initial herbicide applications for the early timing were applied on March 17, 2016 and March 27, 2017 when GDD_{5C} was 200 ± 60. The later application timing occurred on March 29, 2016 and April 4, 2017 when GDD_{5C} was 300 ± 30. GDD_{5C} were enumerated starting Jan 1 each year as has been calculated in other studies (Patton et al., 2004; Rimi et al., 2012; Schiavon et al., 2011; Severmutlu et al., 2011). Both of these timings would be considered later than that normally recommended for nonselective herbicide sprays on dormant turf in Virginia, which coincides with between 50 and 100 GDD_{5C} in early February (Rimi et al., 2012). Since zoysiagrass has been injured by herbicides when treated at later spring timings (Velsor et al., 1989; Xiong et al., 2007) reportedly after the initiation of green-up, these timings were chosen to represent early- and mid-green-up.

Zoysiagrass percentage green cover, turfgrass injury, green leaves per dm^{-2}, and NDVI data were collected at 0, 7, 14, 21, 28, 42, and 56 days after treatment. Zoysiagrass injury was assessed visually on a 0 to 100 % scale, where 0 indicates that plots had equivalent green zoysiagrass vegetation compared to the nontreated and 100 % indicates all green vegetation of the zoysiagrass was eliminated. Injury of 30 % or greater was considered unacceptable injury. Zoysiagrass green cover was assessed as a visually estimated percentage of the plot area. Zoysiagrass green leaf counts were collected by counting all green leaves present within a 10-cm-by-10-cm, randomly chosen location in each plot, counting all leaves within the canopy that were at least half green and extrapolating to dm^{-2}. Measurements of NDVI were collected using a Holland Scientific Crop Circle ACS 210 active crop sensor (Holland Scientific Inc., Lincoln, NE) affixed 43 cm above the turf that collected 50 ± 5 readings per plot that represented a 0.5 x 1.6 m area of turf canopy in the center of each plot.

Zoysiagrass percentage green cover, turfgrass injury, green leaves per dm^{-2}, and NDVI data over time were converted to the area under progress curve (AUPC) using the same formula and parameters as mentioned previously. Zoysiagrass injury maxima was recorded as the maximum value observed at any assessment data. Zoysiagrass NDVI, green cover, and green leaves dm^{-2} were also subjected to linear regression and slopes from each experimental unit were analyzed for treatment effects. The slopes, expressed as the change in response d^{-1}, allow for the estimation trends over time that otherwise would not be evident from AUPC d^{-1} data. Slope and AUPC d^{-1} data for zoysiagrass green cover, NDVI, and green leaves dm^{-2}, along with injury maxima, were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect replication, site, year, and site x year as random effects and herbicide, application timing, and herbicide x application timing as fixed effects. The model included all possible combinations of interactions between the random site, year, and site x year and the fixed effects or interactions. Mean square error associated with herbicide, application timing, and herbicide x application timing were tested with the mean square associated with their interaction with the random variables (McIntosh, 1983). Data were discussed separately by site, year, or site x year if significant interaction was detected ($P < 0.05$). Otherwise, data were pooled over site and or year. Appropriate interactions or main effects were subjected to Fisher's Protected LSD test at $\alpha = 0.05$. The relationship between visually estimated zoysiagrass green cover and zoysiagrass leaves dm^{-2} was further investigated via linear regression.

Results and Discussion

Herbicide and temperature effects on zoysiagrass sprigs

Both glyphosate and glufosinate caused sigmoidal trends in green cover reduction of zoysiagrass sprigs over time (Figures 1 and 2). The colder temperature of 10 °C tended to slow the rate of cover reduction caused by glyphosate (Figure 1) and glufosinate (Figure 2). Glyphosate tended to exhibit a stepwise rate of green cover reduction over time with increasing temperature (Figure 1). For glufosinate, higher temperatures of 18 and 27 °C caused rapid green cover reduction within a few days while the colder temperature 10 °C was slightly slower by comparison (Figure 2). The 10 °C temperature slowed the rate of green cover loss by zoysiagrass sprigs for both herbicides but did not prevent complete or near-complete green cover loss in either case. Although delayed in the cold chamber, green cover loss increased rapidly upon moving sprigs to the warmer greenhouse conditions of 27 °C at seven DAT (Figures 1 and 2).

The interaction of temperature x herbicide was significant for time to GCR_{50} ($P = 0.0225$) and was not dependent on herbicide rate ($P = 0.1217$). Glufosinate reduced zoysiagrass time to GCR_{50} more rapidly than glyphosate regardless of temperature regime (Table 1). The seven day incubation period at 27, 18, and 10 °C caused a stepwise increase in the number of days required to reach GCR_{50} as temperature decreased for both herbicides. The difference between the number of days required for zoysiagrass sprigs to reach GCR_{50} between the 10 °C and 27 °C temperatures is 6.3 and 7.1 days for glyphosate and glufosinate, respectively. Since sprigs were exposed to 10 °C conditions for 7 d, these data suggest cold temperature stalls herbicidal activity, but as temperature warms, herbicidal activity will respond accordingly.

Glufosinate has been shown to injure plants more quickly than glyphosate resulting in reduced translocation of glufosinate (Bromilow et al., 1993; Steckel et al., 1997). Plants typically exhibit herbicide symptoms in 3 to 5 days after glufosinate treatment (Steckel et al., 1997; Pline et al., 1999) and 4 to 7 days after glyphosate treatment (Pline et al., 1999b). The rapid activity of glufosinate is a result of inhibiting glutamine synthetase, and causing a build-up of ammonia in plant cells that depletes the plant of crucial amino acids (Pline et al., 1999; Wendler et al., 1990). Our findings are supported by those of Anderson et al. (1993), who found glufosinate injury to green foxtail was delayed but not substantially reduced by temporary incubation at 8 °C.

The interaction of herbicide by temperature was significant for sprig biomass ($P = 0.0149$) and not dependent on herbicide rate ($P = 0.9832$). Lack of significant differences in biomass between shoots exposed to either herbicide at 10 °C compared to that of 27 °C (Table 1), is further evidence that temperature influences were just a transient effect. Despite a small difference between glufosinate-treated sprigs between 18 and 27 °C, the only biologically significant differences in sprig biomass were between the nontreated controls and herbicide-treated sprigs (Table 1).

Herbicide and temperature effects on plugs of zoysiagrass

To better evaluate the apparent differences between zoysiagrass responses in controlled temperature chambers compared to the warmer greenhouse, the study involving zoysiagrass plugs explored whole-canopy responses after a greater (14-d) temperature incubation period, and the NDVI d^{-1} data were separated by the time spent in the temperature chambers and the subsequent “post-chamber” time spent in the greenhouse (Table 1). The interaction of herbicide x temperature was significant for NDVI in-chamber AUPC d^{-1} (P = 0.0186) and NDVI post-chamber AUPC d^{-1} (P = 0.0038), but neither in-chamber (P = 0.0523) nor post-chamber (P = 0.4155) interactions were dependent on year.

The in-chamber NDVI AUPC d^{-1} for nontreated, glyphosate-treated, and glufosinate-treated turf plugs was statistically similar when turf was exposed to 10 °C (Table 1). These responses differed, however, when temperatures were increased to 18 or 27 °C. At both of these higher temperatures, glufosinate lowered NDVI d^{-1} compared to glyphosate or the nontreated. While plants were in the temperature chambers, nontreated turf exhibited a stepwise increase in NDVI d^{-1} as temperature increased. It has been well documented that zoysiagrass growth increases with increasing temperatures within the temperature range tested (Patton et al., 2004). Research has shown glyphosate and glufosinate injure plants more rapidly as temperatures increase (Jordan, 1977; Pline et al., 1999a). Data from both the sprig and plug studies indicate that glufosinate may be more injurious to zoysiagrass than glyphosate based on speed of activity (Table 1). To better explore this possibility, one can examine the post-chamber NDVI d^{-1} data to evaluate possible differences in turf recovery between the two herbicides that may have occurred over the 42-d incubation period after turf plugs were removed from temperature chambers and transported to the greenhouse at 27 °C.

Zoysiagrass turf did not vary in post-chamber NDVI d^{-1} based on the different temperatures to which they had previously been exposed for any level of herbicide treatment (Table 1). Thus, any lasting effects of the 14-d temperature treatment prior to moving plants to the greenhouse did not alter NDVI d^{-1} during the 42-d post-chamber incubation. This suggests that changes in NDVI d^{-1} during the post-chamber period must have been solely due to herbicide. For all temperatures, glufosinate reduced zoysiagrass turf NDVI d^{-1} more than glyphosate and the nontreated (Table 1).

These data suggest that zoysiagrass was either injured less by or recovered more following glyphosate treatment when compared to glufosinate treatment. Differential zoysiagrass injury between glufosinate and glyphosate has not been previously reported. However, research has shown that low temperatures can affect herbicide absorption and translocation (Duke and Hunt, 1977; Pline et al., 1999a).

Like other variables, the interaction of herbicide by temperature was significant for time to reach GTC_{50} ($P < 0.0001$) and not dependent on year ($P = 0.2311$). When incubated at 10 °C for 14 DAT, the nontreated zoysiagrass plugs and glyphosate-treated plugs both reached 50 % green cover in 22 days compared to 70 days for plugs treated with glufosinate (Table 1). These data suggest that the cold temperature over a 14-d duration may have safened zoysiagrass to glyphosate treatment but not to glufosinate treatment. The similarity between zoysiagrass green cover between nontreated and glyphosate-treated plugs incubated at 10 °C is apparent in the aerial images (Figure 3), and contrasts with that observed when zoysiagrass sprigs were exposed to cold temperatures for only 7 d (Table 1). Post-treatment incubation of zoysiagrass at both higher temperatures caused the herbicides to substantially delay GTC_{50}. In the case of glufosinate-treated zoysiagrass incubated at 27 °C, the time required to reach 50 % green turf cover was increased 18 fold from 3.4 d to 62 d.

Heat unit effects on zoysiagrass response to herbicides

Injury maxima following seven unique herbicide treatments exhibited a significant herbicide by application timing interaction ($P = 0.0336$) but was not dependent on site ($P = 0.0645$), year ($P = 0.4603$), or site x year ($P = 0.0529$). Maximum injury was increased by application timings of ~200 or 300 GDD_{5C} for diquat, glufosinate, glyphosate, and metsulfuron + rimsulfuron but not for flumioxazin, foramsulfuron, or oxadiazon (Table 2). Glufosinate injured zoysiagrass turf more than all other herbicides at both application timings. A common threshold for maximum injury is 30 %, below which most turf managers would be presumed not to take any action to promote quality improvement or turf recovery (Cox et al., 2017). Foramsulfuron and oxadiazon at either application timing and glyphosate at the ~ 200 GDD_{5C} timing did not injure zoysiagrass at or above the 30 % threshold on any of the assessment dates (Table 2). All other treatments would be deemed too injurious to use at application timings of ~ 200 GDD_{5C} or later. This increased zoysiagrass injury by glufosinate compared to glyphosate is supported by results on both sprigs and turf-canopy plugs in controlled-temperature conditions (Table 1). The results, however, seem to contrast partially with a report by Xiong et al. (2013), who reported greater zoysiagrass turf quality reductions at later application timings as in our study, but observed no differences between glyphosate and glufosinate effects on zoysiagrass quality when these herbicides were applied at rates equivalent to our study. Using archived climate data from the National Oceanic and Atmospheric Administration for Columbia, MO and Carbondale, IL, we determined that GDD_{5C} in the Xiong et al. (2013) study was ~ 29 GDD_{5c} for treatments that minimally impacted zoysiagrass and ~ 144 GDD_{5c} for treatments that substantially reduced turfgrass quality. Thus injurious application timings in the Xiong et al. (2013) study was more similar to our early application timing.

The interaction of herbicide x application timing was significant for zoysiagrass turf injury AUPC d^{-1} ($P = 0.0015$) and not dependent on site ($P = 0.0742$), year ($P = 0.0507$), or site x year ($P = 0.2224$). Average injury d^{-1} was higher at the ~300 GDD_{5C} timing than the ~ 200 GDD_{5C} timing for all herbicides except flumioxazin (Table 2). Glyphosate applied at ~ 200 GDD_{5C} maintained injury d^{-1} of 8.3 % and equivalent to the safest herbicides evaluated. At ~ 300 GDD_{5C}, however, glyphosate was much more injurious and averaged 55 % injury d^{-1}. As with injury maxima (Table 2), injury d^{-1} caused by glufosinate was significantly greater than all other herbicides, regardless of application timing. These data suggest that, although applications beyond 100 GDD_{5C} should be dissuaded, glyphosate may be applied more safely over a broader application period than glufosinate.

The interaction of herbicide x application timing for NDVI slopes over time and average NDVI AUPC d^{-1} were significant ($P < 0.05$) and not dependent on site, year, or site x year ($P > 0.05$). Slopes of NDVI over time indicate that nontreated turf gained 0.0085 NDVI d^{-1} , consistent with an increase in zoysiagrass quality (Table 2). Thus, the average daily NDVI based AUPC of 0.5 would have taken approximately 59 days to achieve based on the slope and assuming an intercept of zero. These linear trends in NDVI over time are due to the fact that zoysiagrass turf was mostly brown at study initiation and greened rapidly during the evaluation period. Our goal of applying treatments at early- and mid-green-up are somewhat verified by these trends in NDVI over time. NDVI slopes over time were significantly greater at the earlier application timing for flumioxazin, glufosinate, glyphosate, and metsulfuron + rimsulfuron, likely due to increased injury by these herbicides at the later application timing (Table 2). The average NDVI AUPC d^{-1} also differed between application times for both glufosinate and glyphosate in agreement with injury data (Table 2). Generally, the more injurious herbicide treatments significantly reduced the average NDVI AUPC d^{-1} compared to the nontreated (Table 2).

Like other variables, the interaction of herbicide by application timing was significant for green turf cover slopes over time and green turf cover AUPC d^{-1} ($P < 0.05$) and not dependent on site, year, or site x year ($P > 0.05$). The slope of green turf cover over time for nontreated turf suggests that 1.9 % cover was added each day with estimated complete coverage by about 53 d after initial treatment (Table 2). Slopes of green turf cover over time were reduced relative to the nontreated by glufosinate or metsulfuron + rimsulfuron at either application timing and by glyphosate at the later application timing (Table 2).

The interaction of herbicide by application timing was also significant for green leaves dm^{-2} slopes over time, and green leaves dm^{-2} AUPC d^{-1} ($P < 0.05$) and not dependent on site, year, or site x year ($P > 0.05$). A strong site main effect for green leaves m^{-2} slope over time with $P < 0.0001$ and $F = 4434$ suggest that the magnitude of green leaf accumulation and over time was influence by site, but lack of significant herbicide and timing interactions with site indicate these variables impacted green leaves m^{-2} equivalently with respect to the site. This site effect is most likely due to differences in mowing height between sites. The relationship between zoysiagrass green cover and green leaves m^{-2} is shown in Figure 4. Linear trend lines explain at least 93 % of data variance and indicate that 'Meyer' zoysiagrass maintained at 1.5 cm gains 3.6 new leaves for each one percent increase in green cover while a 'Zenith' + 'Companion' blend maintained at 3.8 cm has 1.8 green leaves for each percentage turf green cover (Figure 4). The leaves of the lawn-height (3.8 cm) zoysiagrass were larger and less abundant in the turf canopy compared to the fairway-height turf. Although linear lines are simple to interpret and had a 0.96 R^2 value for the 'Meyer' zoysiagrass site, the data visually indicate that the intercept of 95 green leaves is overestimated for 'Meyer' turf. Interestingly, these data prove that zoysiagrass can have between 25 to over 50 green leaves dm^{-2} in the canopy prior to any surface green cover being observed. These data support previous reports of subcanopy green leaves in turf that was considered to be "dormant" (Velsor et al., 1985). These data also speak to the validity of visually estimated turf cover assessments as being strongly correlated with actual leaf counts.

The effect of herbicide by application timing on the slope of green leaves dm^{-2} over time indicate that glufosinate lowers green leaves dm^{-2} regardless of application time. Glyphosate and metsulfuron + rimsulfuron reduce the rate of green leaf accumulation only when applied at the later application timing (Table 2). Lack of differences between herbicides that were evident in some other response variables but not evident in the slope of green leaves dm^{-2} may be due to turf recovery by the end of the study. Only the most injurious treatments prevented zoysiagrass from recovery by 56 DAT. Other treatments may have made substantial gains in green leaves dm^{-2} toward the final assessment time, which would have influenced linear regression trends. Another problem that limits green leaf counts from explaining trends in zoysiagrass response to herbicides is that herbicides seldom affect the entire leaf uniformly. In this study, green leaves were counted if half the leaf was considered some shade of green. Variable levels of leaf discoloration by some herbicides and not by others was confounded with related effects on stunting of new leaf production to create variability in green leaf dm^{-2} response to herbicides and application time. This issue affected the average green leaves d^{-1} based on AUPC as well (Table 2). These data were able to distinguish differences in green leaves dm^{-2} d^{-1} only for the most injurious herbicide, glufosinate.

These studies show that zoysiagrass injury from the herbicides tested increases with increasing temperature and increasing number of green leaves in the canopy. Glufosinate was consistently more injurious to zoysiagrass than glyphosate or other herbicides. When temperatures were 10 °C for 7 d following treatment, a delayed effect of glyphosate and glufosinate activity was noted on zoysiagrass sprigs but a 14-d incubation period at 10 °C reduced overall injury by glyphosate but not by glufosinate. When treated at approximately 200 GDD$_{5C}$, zoysiagrass will develop green cover equivalently to nontreated turf following exposure to all herbicides except glufosinate and metsulfuron + rimsulfuron. When treated at approximately 300 GDD$_{5C}$, only foramsulfuron and oxadiazon can safely be used. These data agree with previous reports that zoysiagrass injury increases when nonselective herbicides are applied later in the spring (Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013). We further provided a GDD_{5C} reference for zoysiagrass response to herbicides, described temperature dependencies for speed of activity following glyphosate or glufosinate treatment and showed that glufosinate is more injurious than several other herbicides in contrast to previous reports (Xiong et al., 2013). Our data also show that green leaves dm^{-2} are strongly correlated to visually estimated green cover, dependent on locations characterized by different mowing heights, and present within the canopy even when the upper canopy is completely brown.

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Table 1. Influence of herbicide and temperature on digitally assessed green cover and normalized difference vegetation index (NDVI) of individual sprigs or turf plugs.

		Time to GCR_{50} for Sprigs				Sprig biomass		
Treatment	Rate	10 C	18 C	27 C		10 C	18 C	27 C
	(g ha^{-1})	d				g		
Nontreated		--	--	--		0.0945a	0.1115a	0.1192a
Glyphosate	Avg. n=3	10a	5.1b	2.7c		0.0449a	0.0377a	0.0400a
Glufosinate	Avg. n=3	8.0a	1.7b	0.9c		0.0311ab	0.0386a	0.0303b
LSD (0.05)		1.8	0.8	0.6		0.0355	0.0109	0.0256

		NDVI in-chamber AUPC for Plugs			NDVI post-chamber AUPC for Plugs			Time to GTC_{50} for Plugs		
		10 C	18 C	27 C	10 C	18 C	27 C	10 C	18 C	27 C
		(Avg. d^{-1})			(Avg. d^{-1})			d		
Nontreated		0.2772a	0.3871b	0.5404c	0.6039a	0.6759b	0.7398c	22a	14b	3.4c
Glyphosate	520	0.2437b	0.3362a	0.3743a	0.6402	0.6082	0.5970	22a	24b	19c
Glufosinate	1680	0.2294a	0.2225a	0.1943b	0.3148	0.2942	0.3072	70a	71a	62b
LSD (0.05)		NS	0.0510	0.0437	0.0949	0.0638	0.0584	1.6	1.1	2.5

Means within a given level of herbicide and across the three levels of temperature that are followed by the same letter are not significantly different according to Fisher's Protected LSD Test at $\alpha = 0.05$. For within temperature comparison across herbicides, an LSD is provided under each column of means.

Table 2. Influence of herbicide and application timing (approximately 200 and 300 GDD$_{5C}$) on percentage maximum zoysiagrass injury and both slope over time and area under the progress curve (AUPC) d^{-1} of normalized difference vegetation index (NDVI), percentage green turf cover, and number of green leaves dm^{-2}. Average daily values were based on AUPC following seven assessments over a 56-d period after treatment to semi-dormant zoysiagrass, averaged over four studies conducted at two sites for two years.

		Injury maxima		Injury AUPC		NDVI x Time		NDVI AUPC	
Treatment	Rate	200 GDD	300 GDD	200 GDD	300 GDD	200 GDD	300 GDD	200 GDD	300 GDD
	(g ha^{-1})	(%)	(%)	(Avg. % d^{-1})	(Avg. % d^{-1})	(Δ d^{-1})	(Δ d^{-1})	(Avg. d^{-1})	(Avg. d^{-1})
Nontreated	--	--		--		0.0085		0.5003	
Diquat	560	39*	62*	12*	24*	0.0100†	0.0092	0.4633	0.4307†
Flumioxazin	428	44	40	18	23	0.0101*†	0.0085*	0.4488†	0.4381†
Foramsulfuron	28.9	13	22	4.0*	11*	0.0094	0.0086	0.4816	0.4686
Glufosinate	1680	64*	88*	40*	65*	0.0087*	0.0053*†	0.3889*†	0.3371*†
Glyphosate	520	18*	55*	8.3*	36*	0.0097*	0.0064*†	0.4680*	0.4083*†
Metsulfuron + Rimsulfuron	21.0 + 17.5	40*	62*	22*	39*	0.0077*	0.0058*†	0.4282†	0.4056†
Oxadiazon	3383	16	24	4.3*	11*	0.0089	0.0086	0.4784	0.4677
LSD (0.05)		13	11	6.1	7.8	0.0012	0.0013	0.0413	0.0447
		Green cover (%) x Time		Green cover AUPC		Green leaf dm^{-2} x Time		Green leaf dm^{-2} AUPC	
		200 GDD	300 GDD	200 GDD	300 GDD	200 GDD	300 GDD	200 GDD	300 GDD
		(Δ d^{-1})	(Δ d^{-1})	(Avg. % d^{-1})	(Avg. % d^{-1})	(Δ d^{-1})	(Δ d^{-1})	(Avg. # d^{-1})	(Avg. # d^{-1})
Nontreated	--	1.90		52		5.19		227	
Diquat	560	1.97*	1.88*	48	43†	5.47	5.07	208	190
Flumioxazin	428	1.92*	1.79*	48	43†	5.48	4.88	203	198
Foramsulfuron	28.9	1.94	1.87	51	48	5.22	4.96	223	215
Glufosinate	1680	1.68*†	1.04*†	35*†	21*†	4.82*	2.73*†	158†	125†
Glyphosate	520	1.87*	1.40*†	49*	36*†	5.06	4.11†	212	188
Metsulfuron + Rimsulfuron	21.0 + 17.5	1.66*†	1.29*†	41†	33†	4.66	3.91†	198	180
Oxadiazon	3383	1.90	1.86	51	49	5.17	5.16	224	214
LSD (0.05)		0.08	0.14	8.9	9.1	NS	1.31	NS	66.8

Means followed by a * were significantly different between herbicide application timings. Means followed by a † were significantly different compared to the nontreated based on single-degree-of-freedom comparisons.

Figure 1. Average effect of three glyphosate rates on green cover reduction of zoysiagrass sprigs relative to nontreated sprigs over time as influenced by exposure to three constant temperature regimes for the first seven days after treatment. All sprigs were exposed to 27 C starting 7-d after treatment.

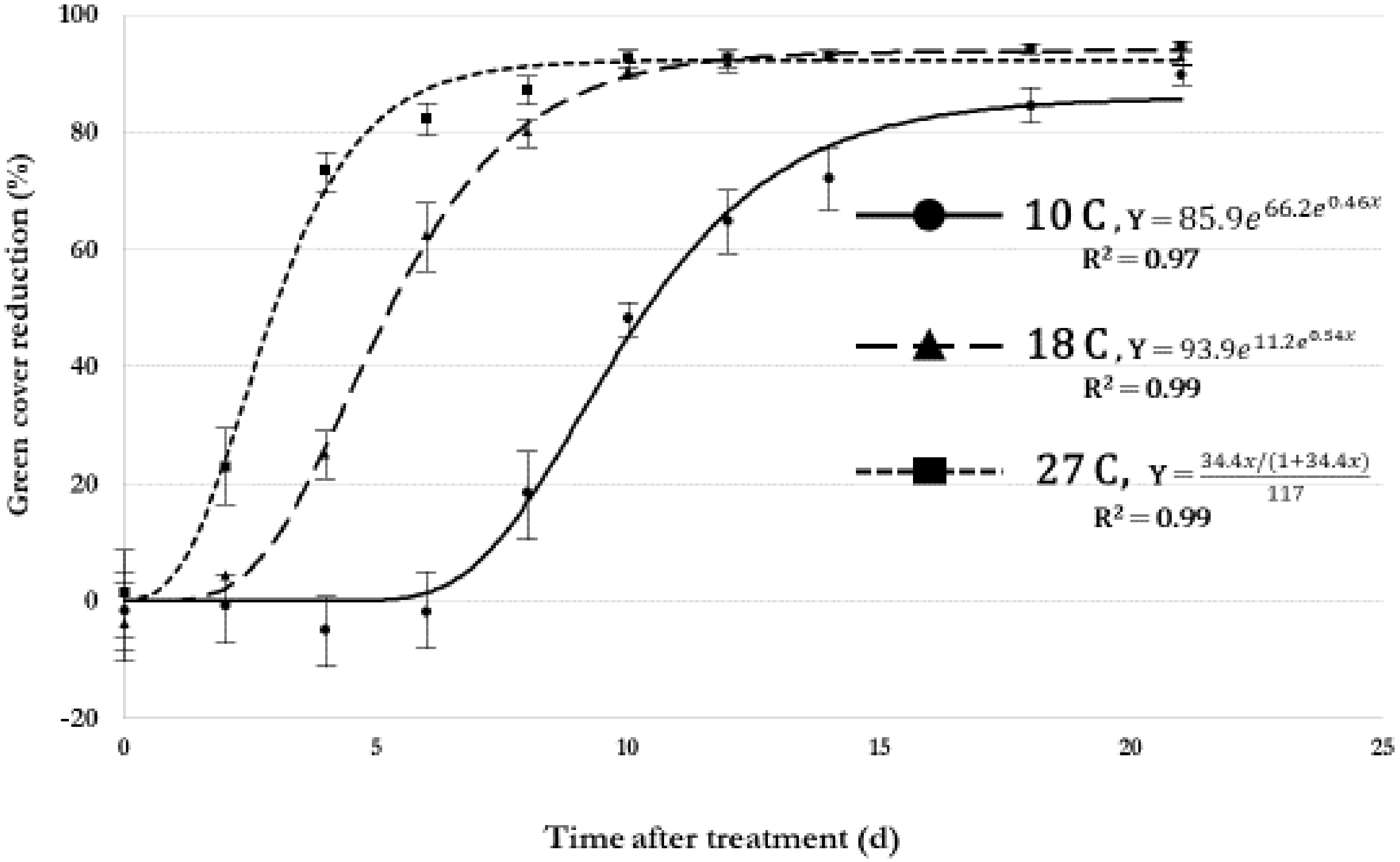


Figure 2. Average effect of three glufosinate rates on green cover reduction of zoysiagrass sprigs relative to the nontreated over time as influenced by exposure to three constant temperature regimes for the first seven days after treatment. All sprigs were exposed to 27 C starting 7-d after treatment.

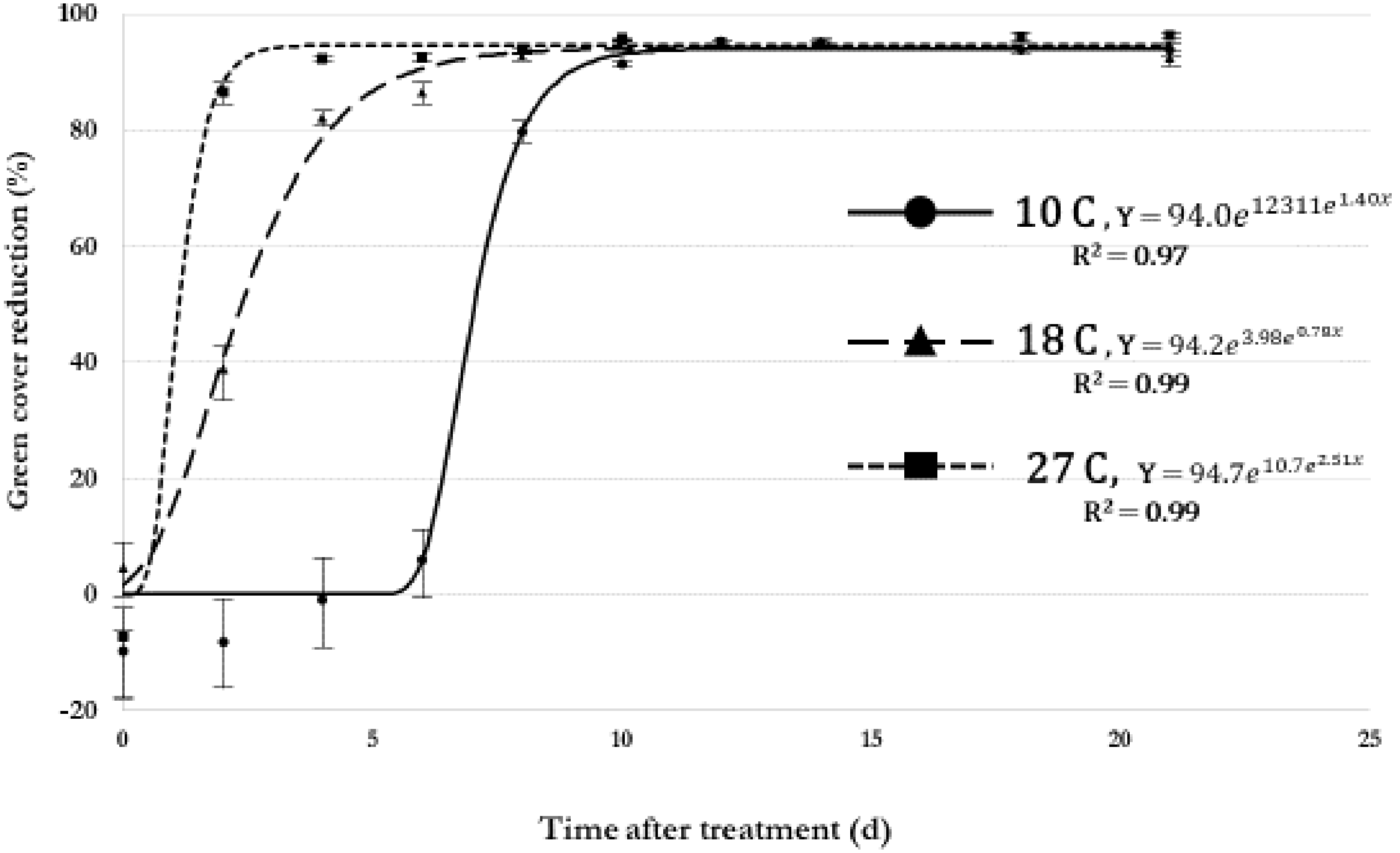


Figure 3. One of eight replicates showing a selection of assessment dates between 0 and 42 days after treatment (DAT) to demonstrate the effects of herbicide and temperature on zoysiagrass green turf cover over time when 10-cm turf plugs were treated at approximately 5 % green cover.

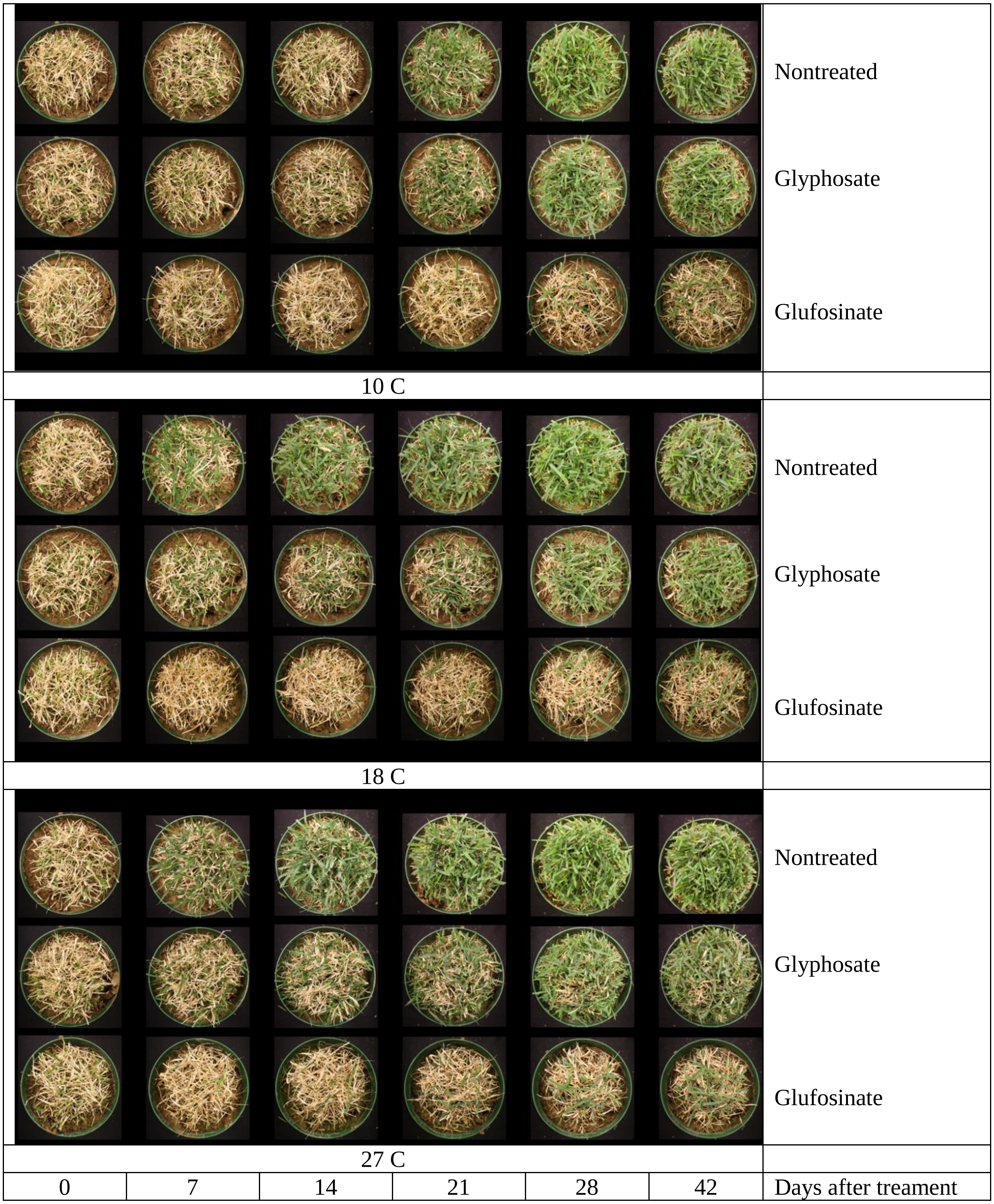
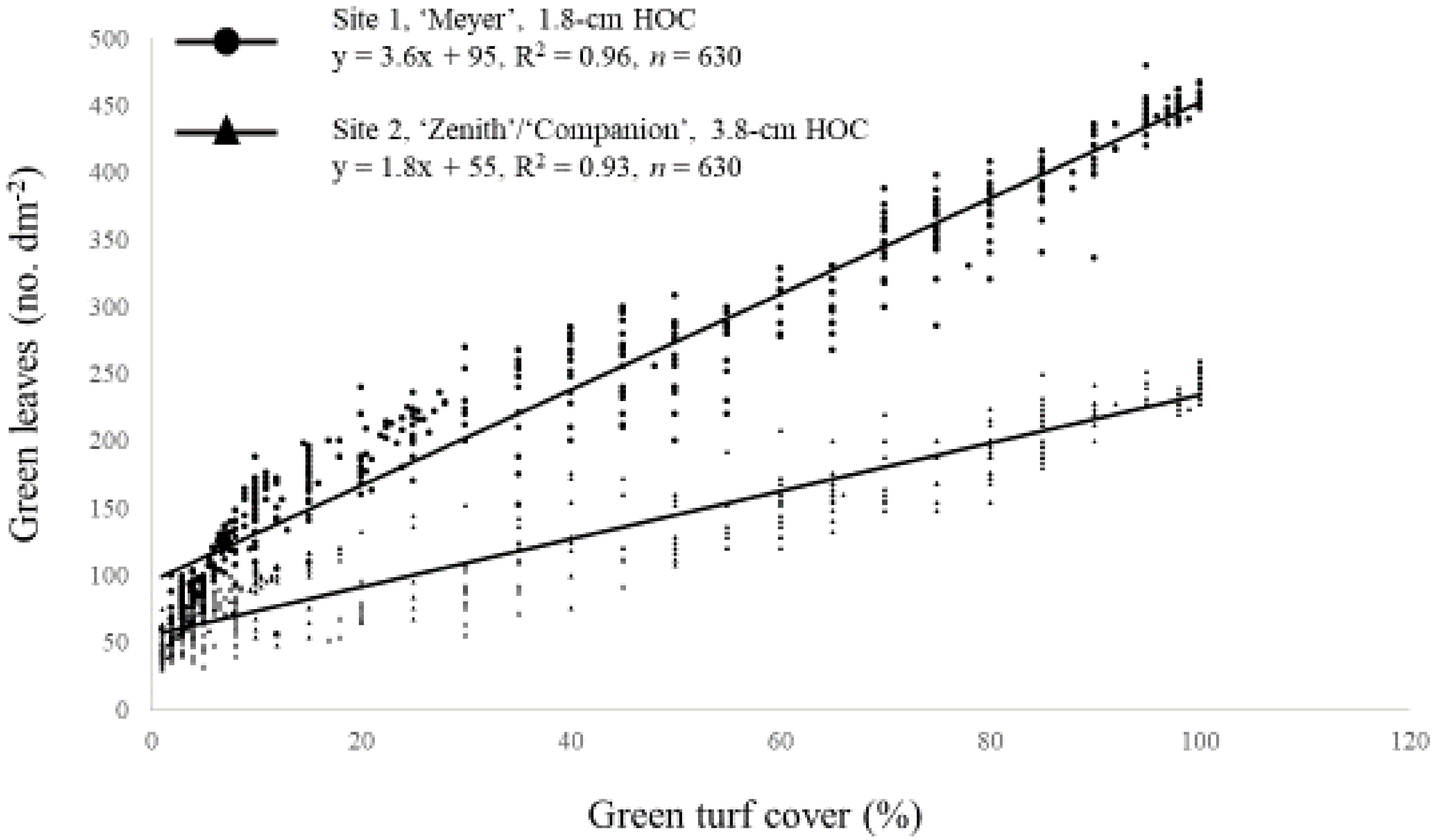


Figure 4. Relationship between visually estimated zoysiagrass green cover and green leaves dm^{-2} from studies conducted over two years at two sites spatially separated by 0.1 km and characterized by different zoysiagrass varieties and mowing heights of cut (HOC).



Chapter 4. Regional response of zoysiagrass turf to glyphosate and glufosinate applied based on accumulated heat units

Abstract

Zoysiagrass has been shown to retain green leaf and stem tissue during winter dormancy, especially in southern regions. Concerns about partially-green zoysiagrass often deter the use of nonselective herbicides. The objective of this study was to evaluate zoysiagrass turf response to glyphosate and glufosinate when applied at four growing-degree-day-based application timings during the outset of spring green-up and to determine if responses varied between Blacksburg, VA; Starkville, MS; and Virginia Beach, VA in 2018 and 2019. Growing-degree-days were calculated using a 5 °C base temperature with accumulation beginning on January 1 in each year, and targeted application timings were 125, 200, 375, and 450 GDD_{5C}.

Zoysiagrass response to glyphosate and glufosinate was consistent across a broad growing region from Northern Mississippi to Coastal Virginia but varied by application timing. Glyphosate and glufosinate exhibited a stepwise increase in maximum injury with increasing targeted GDD_{5C} application timings. Glyphosate applied at 125 and 200 GDD_{5C} did not injury zoysiagrass above a threshold of 30 %, while glufosinate injured zoysiagrass greater than 30 % and was more injurious than glyphosate at all application timings. Increased injury caused when herbicides were applied at greater cumulative heat units may have been promoted by green leaf density, which was strongly related to heat unit accumulation.

Nomenclature: Zoysiagrass, *Zoysia* spp.; GDD, growing-degree-days.

Keywords: Zoysiagrass, dormancy, turfgrass injury, glyphosate, glufosinate, heat units, growing-degree-days.

Introduction

Turfgrasses, such as zoysiagrass (*Zoysia* spp.), are adapted to various growing regions based on temperature (Patton et al., 2004). Accumulated heat units or growing-degree-days (GDD) have been widely used to estimate crop productivity (Major et al., 1983), predict phenological development of weeds (Miller et al., 2001), and trigger pesticide applications (Dale and Renner, 2005; Forcella and Banken, 1996). Variation in temperatures between seasons is said to be the primary influence on corn productivity, assuming moisture and fertility needs are met (Major et al. 1983). In turfgrass, GDD models have been used to optimize growth regulators, insecticide, and herbicide application intervals, and to predict seedhead development, weed emergence, and disease occurrence (Brosnan et al., 2010; Danneberger et al., 1987; Fidanz et al., 1996; Kreuser and Soldat, 2011; McCullough et al., 2017; Reasor et al., 2018; Ryan et al., 2012). Researchers have developed GDD models for zoysiagrass establishment, but none have examined the relationship between GDD accumulation and zoysiagrass green-up (Patton et al., 2004; Sladek et al., 2011).

Winter applications of nonselective herbicides for weed control are a common practice in dormant bermudagrass (*Cynodon dactylon* L.) (Johnson, 1976; Rimi et al., 2012; Toler et al., 2007). However, turfgrass managers are often hesitant to make these applications to dormant zoysiagrass due to fear of injuring and delaying spring green-up (Boyd, 2016; Brosnan and Breeden, 2011). Variable responses of both bermudagrass and zoysiagrass have been reported following glyphosate or glufosinate treatment at various stages of post-dormancy green-up in spring (Johnson, 1976; Johnson and Ware, 1978; Xiong et al., 2013). All of the research examining zoysiagrasses' response to nonselective herbicides has been conducted in upper climatic-transition zones with northern latitudes between 37 and 45 where zoysiagrass is more likely to be fully dormant (Hoyle and Reeves, 2017; Rimi et al., 2012; Veslor et al. 1989; Xiong et al. 2013).

The research focusing on zoysiagrass has indicated that application time is a critical factor influencing zoysiagrass safety. However, the description of application timings are often described as the time before green-up or based strictly on calendar dates (Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013). Velsor et al. (1989) reported that glyphosate applied on April 1 caused significant injury, but the same application on March 1 caused no zoysiagrass injury. Xiong et al. (2013) observed zoysiagrass injury from glyphosate and glufosinate when applied “2 to 3 days before green-up” but not when applied “2 to 3 weeks before green-up.”

Development of zoysiagrass based on GDD has been reported (Patton et al., 2004) but not across broad geographies. Although zoysiagrass response to glyphosate and glufosinate has differed based on accumulated GDD in Virginia (unpublished data) and in Italy (Rimi et al., 2012), regional differences in factors such as seasonal precipitation legacy (Shen et al., 2015) and winter severity (Schwab et al., 1996) have altered plant responses between locations. The objective of this study was to evaluate zoysiagrass turf response to glyphosate and glufosinate when applied at four GDD-based application timings during the outset of spring green-up and to determine if responses varied between Blacksburg, VA; Starkville, MS; and Virginia Beach, VA.

Materials and Methods

Four trials were initiated in the spring of 2018 and repeated in 2019 in Blacksburg and Virginia Beach, VA, and Starkville, MS, to zoysiagrass response to GDD based application timings of glyphosate and glufosinate. Two trial sites were conducted at the Virginia Tech

Turfgrass Research Center in Blacksburg, VA (37.214472, -80.411476). One trial site was a mature stand of ‘Meyer’ (*Z. japonica*) zoysiagrass mown with a reel mower at 1.5 cm during active growth. The second trial site was a mixed stand of ‘Zenith’ (*Z. japonica*) and ‘Companion’ (*Z. japonica*) zoysiagrass mown with a rotary mower at 6.5 cm during active growth. The soil type at both locations was Groseclose urban loam (clayey, mixed, mesic, Typic Hapludalfts) with a pH of 6.2 and organic matter ranging from 2.8 to 4.1 %. Irrigation, fertility, and pesticide applications were withheld from the trial sites during the experiment. Another trial site was conducted at the Virginia Tech Hampton Roads Agricultural Research and Extension Center in Virginia Beach, VA (36.892052, -76.178496), on a mature stand of ‘Compadre’ (*Z. japonica*) zoysiagrass mown with a rotary mower at 6.3 cm during active growth. The soil type was a Tetolum loam (fine-loamy, mixed, termic Aquic Hapludult) with a pH of 5.4 and an organic matter content of 2.9 %. In 2018, the site had heavy weed pressure making it difficult to evaluate zoysiagrass green-up. Therefore, in 2019, 2, 4-D 2-Ethylhexyl ester + mecoprop-p acid + dicamba acid + carfentrazone-ethyl (Speedzone herbicide, PBI Gordon, Shawnee, KS) at 420 g ai ha^{-1} and flazasulfuron (Katana herbicide, PBI Gordon, Shawnee, KS) at 26.3 g ai ha^{-1} were applied in January to control winter annual weeds to ensure the site had a uniform zoysiagrass stand for the duration of the trial. Another trial location was the Mississippi State University Foil Plant Science Research Center in Starkville, MS (33.470426, -88.779074), on a mature stand of ‘Meyer’ zoysiagrass mown with a reel mower at 1.9 cm during active growth. The site contained a native Marietta fine sandy loam (fine-loamy, siliceous, active, Fluvaquentic Eutrudept) soil with a pH of 6.2. Irrigation, fertility, and pesticide applications were withheld during the experiment.

All experiments were arranged as a randomized complete block design with four replications with a two (herbicide) by four (timing) factorial arrangement of treatments. Plots measured 1.8 by 1.8 m at the Blacksburg and Starkville sites and 1.8 by 10 m at the Virginia Beach site. Herbicide treatments included glyphosate (Roundup Pro Concentrate herbicide, Bayer Environmental Sciences, Research Triangle Park, NC) at 520 g ae ha^{-1} and glufosinate (Finale herbicide, Bayer Environmental Sciences, Research Triangle Park, NC) at 1680 g ai ha^{-1}. Herbicide rates were based on rates that had been shown to control annual bluegrass (*Poa annua* L.) in late winter and early spring. Herbicide treatments were applied at all sites using CO_2 powered boom sprayers equipped with TTI nozzles (Teejet Technologies, Springfield, IL) calibrated to deliver 280 L ha^1. Growing-degree-days were calculated daily using a 5 °C base temperature beginning on Jan 1 as in other studies (McMaster and Wilhelm, 1997; Patton et al., 2004). Targeted application timings were 125, 200, 275, and 350 GDD_{5C}. Actual accumulated GDD across the eight site years varied due to factors such as inclement weather and were 126 ± 60, 192 ± 75, 256 ± 72, and 337 ± 44.

The number of green zoysiagrass leaves dm^{-2} were counted prior to each treatment by randomly choosing a 10-cm-by-10-cm area, counting all leaves within the canopy that were at least half green and extrapolating to dm^{-2}. Zoysiagrass injury was assessed visually on a 0 to 100 % scale, where 0 indicates that plots had equivalent green zoysiagrass vegetation compared to the nontreated and 100 % indicates all green vegetation of the zoysiagrass turf was eliminated. Injury of 30 % or greater was considered unacceptable turfgrass injury. Zoysiagrass green cover was assessed as a visually estimated percentage of the plot area. Measurements of normalized difference vegetation index (NDVI) were collected at the six site years associated with Blacksburg, VA and Starkville, MS using a Holland Scientific Crop Circle ACS 210 active crop sensor (Holland Scientific Inc., Lincoln, NE) affixed 43 cm above the turf that collected 50 ± 5 readings per plot that represented a 0.5 x 1.6 m area of turf canopy in the center of each plot at Blacksburg, VA and a Holland Scientific RapidScan CS45 handheld crop sensor (Holland Scientific Inc.) held 110 cm above and perpendicular to the canopy to scan three 1-m long transects along the center of each plot at Starkville, MS. Assessments were made at 0, 7, 14, 21, 28, 42, 56, 70, 84, 98, and 112 days after initial treatment (DAIT).

Data Analysis

Maximum observed turfgrass injury was reported as the highest injury data recorded on any assessment date. Visually estimated zoysiagrass injury data from the 11 assessment dates were used to calculate the number of days over threshold of 30 % (DOT_{30}) following each application to assess the duration of unacceptable turf injury (Cox et al., 2017). The DOT_{30} were calculated by subjecting observed injury over time from all combinations of the eight site years, application time, herbicide treatment, and replicates to the Gaussian function:

$$y = ae^{\left(\frac{-(x-b)^2}{2c^2}\right)}, \qquad [1]$$

where a is maximum injury, b is the number of d after treatment at which maximum injury occurred, and c is one standard deviation from b. The parameter c can be multiplied by 6 to determine the number of days comprising 3 standard deviations, an approximation of the duration of injury. Fit of the curve was based on least sums of squares using the Gauss-Newton method of PROC NLIN in SAS 9.2 (SAS Institute, Cary, NC). The output from PROC NLIN was then subjected to a logical operation in SAS 9.2 using parameters a and c from equation 1 as follows:

```
if a < 30 then Do;
DOT30 = 0;  End;
Else DOT30 = 2*(sqrt(2*(Log(1/((a-30)/a)))))*c;          [2]
```

Zoysiagrass percentage green cover and NDVI data over time were converted to the area under progress curve (AUPC) (Eq. 3) using

$$\partial = \sum_{i=1}^{ni-1} \left(\frac{(y_i + y_{(i-1)})}{2} (t_{(i+1)} - t_{(i)} \right), \qquad [3]$$

where ∂ is the AUPC, *i* is the ordered sampling date, ni is the number of sampling dates, *y* is turf green cover or NDVI measurements at a given date, and *t* is the time in days. The AUPC was then converted to the average d^{-1} by dividing by the number of days spanned by the assessment period. Campbell and Madden (Campbell and Madden, 1990, p. 192–193) applied this equation to disease epidemiology, and Askew et al. (2013) and Brewer et al. (2017) utilized it for weediness over time in a turfgrass comparison study. The AUPC is useful in situations where long durations are assessed by repeated measures. Zoysiagrass green cover and NDVI data over time were also subjected to linear regression, and slopes from each experimental unit were analyzed for treatment effects. The slopes, expressed as the change in response d^{-1}, allow for the estimation of trends over time that otherwise would not be evident from AUPC d^{-1} data. Slope and AUPC d^{-1} data for zoysiagrass green cover and NDVI along with injury maxima and DOT_{30} were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect replication, site, year, and site x year as random effects and herbicide, application timing, and herbicide by application timing as fixed effects. The model included all possible combinations of interactions between the random site, year, and site by year and the fixed effects or interactions. Mean square error associated with herbicide, application timing, and herbicide by application timing were tested with the mean square associated with their interaction with the random variables (McIntosh, 1983). Data were discussed separately by site, year, or site by year if significant interaction was detected ($P < 0.05$). Otherwise, data were pooled over site and or year. Appropriate interactions or main effects were subjected to Fisher's Protected LSD test at α = 0.05. The relationship between accumulated GDD_{5C} and zoysiagrass leaves dm^{-2} was further investigated via linear regression (Figure 1). An additional data set from four previously conducted studies in Blacksburg, VA (Craft, 2021) where numerous green leaf counts were taken was combined with associated GDD_{5C} that had accumulated at each assessment date and included in the regression. These data were separated by mowing height, and each regression consisted of six site years and 546 observations.

Results and Discussion

The herbicide by application timing interaction was significant ($P = 0.0002$) and not dependent on year ($P = 0.0671$), location ($P = 0.2028$), or year by location ($P = 0.2478$) for maximum zoysiagrass injury, so data were pooled over seven of the eight site years for presentation in Table 1. Zoysiagrass response data for one of the two years at Virginia Beach were confounded by disease pressure and not included in the analysis. Glufosinate was more injurious than glyphosate regardless of application timing and both herbicides exhibited a stepwise increase in maximum injury with increasing targeted GDD_{5C} application timings. With each 75 accumulated GDD_{5C}, maximum zoysiagrass injury increased by approximately 20 % regardless of herbicide (Table 1). These data indicate that glufosinate applied at the maximum label-recommended rate, is more injurious to zoysiagrass than glyphosate and should only be applied under conditions of complete zoysiagrass dormancy. Our results agree with previous reports that zoysiagrass is injured more by glyphosate and glufosinate when the herbicides are applied later in the spring season (Rimi et al., 2012; Velsor et al., 1989). Although maximum injury is important, it does not indicate the duration of injury response. Data from the eleven assessments made over a 112-d period were used to estimate the DOT_{30} for this reason.

Like a maximum injury, the DOT_{30} data were dependent on an interaction of herbicide and application timing ($P < 0.05$) but not dependent on year, location, or year by location ($P > 0.05$). Glyphosate did not injure zoysiagrass above a threshold of 30 % when applied at target timings of 125 and 200 GDD_{5C} and only resulted in an estimated 1.6 d over the 30 % threshold when applied at 275 GDD_{5C}. Glufosinate increased DOT_{30} compared to glyphosate at every application timing (Table 1). It is not surprising that both glyphosate and glufosinate injured zoysiagrass above a 30 % threshold for 36 or 46 d, respectively, when applied at 350 GDD_{5C} as previous reports indicate that the herbicides are more injurious after zoysiagrass has started to green-up (Velsor et al., 1989; Xiong et al., 2013). What's more concerning is the DOT_{30} of 28 caused by glufosinate when applied at 125 GDD_{5C}. These data suggest that glufosinate may injure zoysiagrass even when applied closer to full dormancy as defined by no green leaves or stems found within the zoysiagrass canopy. It should be noted that injury from such early applications may not be detectable by turf managers unless nontreated test strips or accidental sprayer skips are evident. At the 125 GDD_{5C} target application timing, zoysiagrass turf had no more than 2 % green cover at any site and 8 to 48 predominately-subcanopy, green leaves dm^{-2} (Figure 1). At the latest application timing that was targeted for 350 GDD_{5C}, zoysiagrass green leaves dm^{-2} were mowing height dependent (Figure 1). At the four site years where zoysiagrass was mown at 1.9 cm, polynomial regression estimates that turf had 237 green leaves dm^{-2} at 350 GDD_{5C}. When zoysiagrass was mown at 6.5 cm at the other four site years, turf had 91 green leaves dm^{-2} (Figure 1). Previous research in Blacksburg, VA conducted on turf mown within the same height ranges indicates that the 237 green leaves dm^{-2} at 1.9 cm would result in 39 % green turf and the 91 green leaves at 6.5 cm would result in 20 % green turf cover (Craft, 2021). These estimates agree with actual observed green cover at the final application timing, which averaged 49 % and 18 % over the four site years each for turf maintained at 1.8 and 6.5 cm, respectively (data not shown).

The interaction of herbicide and application timing was significant for average turf green cover AUPC d^{-1} ($P = 0.0002$) and not dependent on year, location, or year by location ($P > 0.05$). The nontreated plots across all sites averaged 47 % cover d^{-1} but it is important to note that green cover was initially less than 5 % and increased over time to reach near 100 % cover at the last assessment date. The value of 47 % average cover d^{-1} in nontreated plots allows for comparison between treatments using data that capture all of the variances across 11 assessments but it does not approximate the actual daily cover levels over the 112-d assessment period. Glyphosate did not reduce turf cover AUPC d^{-1} when applied at targeted GDD_{5C} of 125 or 200 in contrast to later application timings where cover AUPC d^{-1} was reduced. Glufosinate reduced turf cover AUPC d^{-1} regardless of application timing but the reductions increased with increasing cumulative GDD_{5C}. Glyphosate and glufosinate have been reported to reduce zoysiagrass “green-up” in other studies when the herbicides were applied at later application timings (Velsor et al., 1989; Xiong et al., 2013).

Turf green cover typically exhibited a linear response over time, but the speed of green cover increase varied between locations and years. The interaction of location by year by herbicide by application timing was significant for slopes of green cover over time ($P = 0.0004$). Data were therefore separated by years and locations and these were further labeled to indicate zoysiagrass height of cut at each location (Table 2). The interaction was likely caused by variable rates of green cover accumulation between years and between warmer locations (Starkville, MS and Virginia Beach, VA) versus cooler locations (the two sites in Blacksburg, VA). For example, the slope of green cover over time in nontreated turf at Starkville, MS was similar to that of Blacksburg, VA in 2018 but seemingly higher in 2019 (Table 2). Only one year could be assessed at Virginia Beach due to issues related to disease, but the data from 2018 appeared to have larger slopes than either year in Blacksburg, VA. Since year and location were random variables, statistical comparisons between these variables were not assessed. We can only speculate about apparent trends in these data. Despite the interaction, some trends were reasonably consistent across the seven assessed site years. Glufosinate and glyphosate, for example, reduced slopes of green cover over time at the two latest application timings in 24 of 28 comparisons (Table 2). Likewise, glyphosate applied at the two earliest timings did not reduce turf cover slopes over time in 12 of 14 comparisons. Glufosinate applied at the two earlier timings was more variable with eight instances of reduced green cover slope and six instances of no impact on green cover slope (Table 2). Green cover slopes over time in nontreated turf ranged from 1.11 % d^{-1} to 1.34 % d^{-1}. When including all 288 experimental units, the intercept averaged 1.03 ± 0.27 (data not shown), so simply multiplying the slope by the number of days and subtracting one yield an approximation of actual green turf cover. Thus, this simple math can be applied to the nontreated turf maintained at 6.5 cm height of cut in Blacksburg, VA in 2018 to show that it required 89 days to reach complete turf cover. When treated with glufosinate at a targeted 350 GDD_{5C}, green cover at this site would have been only 54 % at the end of the 112-d assessment period. In Starkville, MS in 2019, we can calculate that complete cover of nontreated turf was reached in approximately 74 d, and this was increased to 94 d following glufosinate treatment at 350 GDD_{5C} (Table 2).

Addition of herbicide treatments that caused injury typically reduced turf cover and, by association, NDVI. Figure 2 shows that NDVI was 91 % correlated to turf cover with an intercept of 0.2197 NDVI at near-zero turf cover and a slope of 0.0053 NDVI per unit increase in percentage turf green cover (Figure 2). This trend is independent of locations and years as the regression in

Figure 2 consists of over 2000 assessments across six of the eight site years (NDVI not taken at Virginia Beach, VA) and 11 assessment dates. Likewise, the interaction of herbicide by application timing for average NDVI AUPC d^{-1} was significant ($P = 0.0070$) and not dependent on year, location, or year by location ($P > 0.05$). Thus, data were pooled over the six site years for comparison (Table 1). Herbicide and application timing effects on average NDVI AUPC d^{-1} tended to mirror trends in turf green cover AUPC d^{-1} with one exception. When compared to nontreated turf, average NDVI AUPC d^{-1} was not reduced by glyphosate at the earliest application timing, while average turf green cover AUPC d^{-1} was not reduced by glyphosate at the first two application timings (Table 1). We can only speculate that NDVI data accounted for herbicide injury that was imperceptible as change in green turf cover at the second application timing targeted at 200 GDD_{5C}. Glufosinate, however, consistently reduced both turf cover AUPC d^{-1} and NDVI AUPC d^{-1} compared to glyphosate at all application timings (Table 1).

These data suggest that glufosinate is more injurious to zoysiagrass when applied during spring green-up than glyphosate and should only be applied if zoysiagrass is fully dormant and no green material is present within the canopy. Both glyphosate and glufosinate were more injurious to zoysiagrass when applied at later timings due to more green cover being present regardless of growing region. The correlation between green leaves dm^{-1} and GDD accumulation indicates that GDD can be used to target application timing for nonselective applications in dormant zoysiagrass. Results indicate that glyphosate can be applied at 125 and 200 GDD_{5C} safely to zoysiagrass regardless of growing regions between northern Mississippi and Virginia.

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Table 1. Influence of herbicide and GDD-based application timing on injury maxima, Days over threshold of 30% injury (DOT_{30}), green cover, NDVI, and visually-estimated injury expressed as area under the progress curve d^{-1} and slope based on 11 assessments over a 112-d period averaged over eight site years from Blacksburg, VA, Starkville, MS, and Virginia Beach, VA in 2018 and 2019.

Targeted application time (GDD_{5C})	Injury maxima		DOT_{30}	
	Glyphosate	Glufosinate	Glyphosate	Glufosinate
	——— % ———		——— d ———	
125	16*	39*	0.0*	28*
200	23*	58*	0.0*	29*
275	38*	71*	1.6*	44*
350	58*	87*	36*	46*
LSD (0.05)	7.4	6.0	3.6	3.0

	Turf cover AUPC			Turf NDVI AUPC		
	Nontreated	Glyphosate	Glufosinate	Nontreated	Glyphosate	Glufosinate
	——— (Avg. d^{-1}) ———			——— (Avg. d^{-1}) ———		
125	47	46*	43*†	0.4924	0.4808*	0.4639*†
200	--	44*	37*†	--	0.4727*†	0.4373*†
275	--	39*†	29*†	--	0.4693*†	0.4169*†
350	--	35*†	22*†	--	0.4430*†	0.3873*†
LSD (0.05)		2.8	2.8		0.0093	0.0178

Means followed by a * were significantly different between herbicide application timings. Means followed by a † were significantly different compared to the nontreated based on single-degree-of-freedom comparisons.

Table 2. Influence of herbicide and GDD-based application timing on linear slopes of green zoysiagrass turf cover over time based on 11 assessments over a 112-d period averaged over eight site years from Blacksburg, VA, Starkville, MS, and Virginia Beach, VA in 2018 and 2019.

	Turf green cover x time at 1.8 cm height of cut											
Targeted	Blacksburg, VA						Starkville, MS					
application	Nontreated		Glyphosate		Glufosinate		Nontreated		Glyphosate		Glufosinate	
time (GDD$_{5C}$)	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
	——————— (Δ d^{-1}) ———————											
125	1.24	1.16	1.18	1.18*	1.11†	1.09*†	1.15	1.34	1.17	1.32	1.17	1.39
200	--	--	1.15*	1.18*	0.97*†	1.05*†	--	--	1.13*	1.33	1.27*	1.35
275	--	--	1.09*†	1.12*	0.62*†	0.96*†	--	--	1.16*	1.31	0.83*†	1.30
350	--	--	0.95*†	0.68*†	0.36*†	0.50*†	--	--	0.96*†	1.23†	0.69*†	1.06†
LSD (0.05)			0.12	0.06	0.09	0.06			0.10	NS	0.17	0.15

	Turf green cover x time at 6.5 cm height of cut								
Targeted	Blacksburg, VA						Virginia Beach, VA		
application	Nontreated		Glyphosate		Glufosinate		Nontreated	Glyphosate	Glufosinate
time (GDD$_{5C}$)	2018	2019	2018	2019	2018	2019	2018	2018	2018
	——————— (Δ d^{-1}) ———————								
125	1.11	1.15	1.11*	1.15*	1.06*†	1.07*†	1.26	1.32	1.30
200	--	--	1.04†	1.12	0.99†	1.12	--	1.21	1.10†
275	--	--	0.98*†	1.03*†	0.77*†	0.95*†	--	0.76†	0.68†
350	--	--	0.93*†	0.81*†	0.49*†	0.69*†	--	0.40†	0.20†
LSD (0.05)			0.04	0.03	0.10	0.05		0.22	0.20

Means followed by a * were significantly different between herbicide application timings. Means followed by a † were significantly different compared to the nontreated based on single-degree-of-freedom comparisons.

Figure 1. Influence of cumulative growing degree days at base 5 °C on number of zoysiagrass green leaves dm^{-2} from 12 site years comprised of two Blacksburg, VA, sites each conducted in 2016 and 2017 and two Blacksburg, VA, one Starkville, MS , and one Virginia Beach, VA site each conducted in 2018 and 2019. The data are split equally such that 6 sites were maintained at 1.9-cm height of cut (HOC) and 6 sites that were maintained at 6.5-cm HOC. Only nontreated and non-injurious treatments are included.

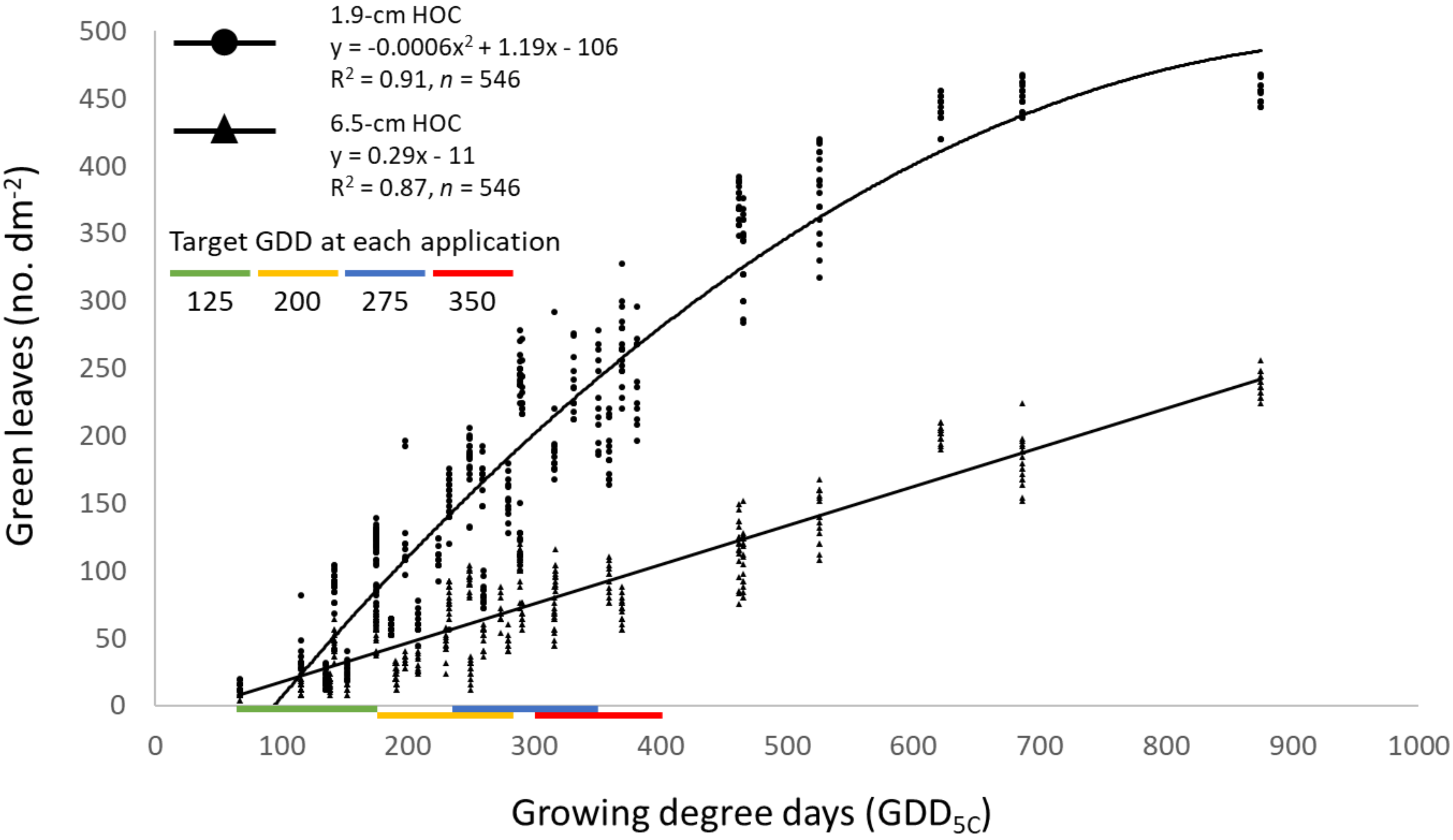
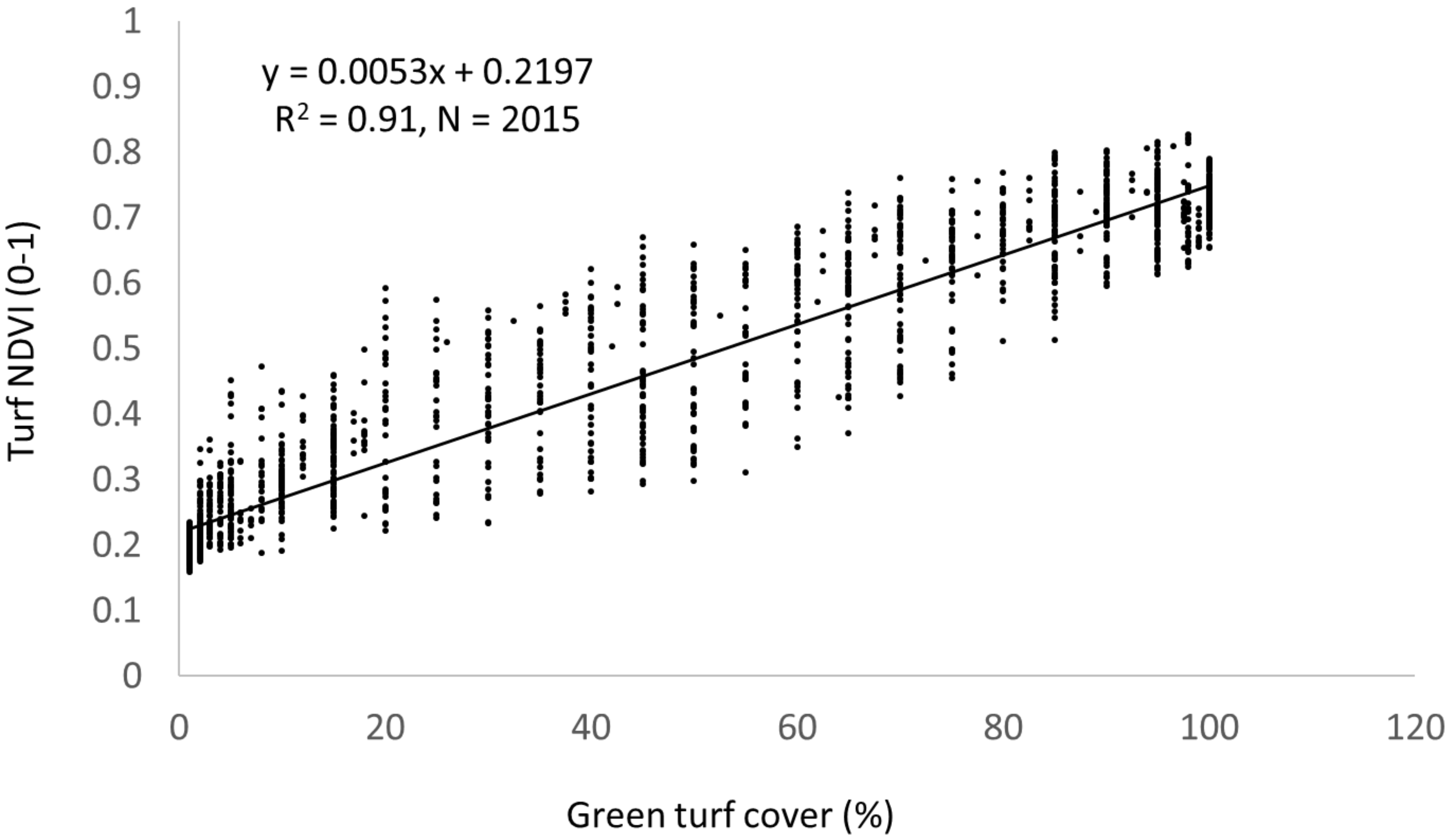


Figure 2. Relationship between normalized difference vegetative index and percentage green cover of zoysiagrass turf from 2015 observations made across six site years at Blacksburg, VA, and Starkville, MS in 2018 and 2019.



Chapter 5. Spray deposition and herbicide absorption in semidormant zoysiagrass

Abstract

Turfgrass managers are concerned about potential turfgrass injury when applying nonselective herbicides to zoysiagrass because sporadic green leaves and stems can be found within the subcanopy almost anytime during winter dormancy. The first objective of this research was to determine the effect of nozzle type, nozzle height above the target, and spray pressure on droplet size and velocity and the penetration of spray droplets into a zoysiagrass turf canopy. The second objective was to evaluate absorption and translocation of ^{14}C-glyphosate and ^{14}C-glufosinate in 'Meyer' zoysiagrass when applied to green leaves or subtending stolons selected from a semidormant canopy.

Increasing pressure from 103 to 414 kPa increased droplet velocities from XR and TTI nozzles and increased droplet diameters of XR nozzles. Droplet diameters were also substantially increased when using TTI nozzles compared to that of XR nozzles. When XR nozzles were positioned 61 cm above zoysiagrass turf, 73 % of recovered colorant was extracted from the upper canopy and 11 % was extracted from the lower canopy based on fluid extraction and spectrophotometric analysis of three 1.9-cm canopy partitions. By comparison, all other nozzle configurations deposited less droplets into the upper canopy and more droplets into the lower canopy. Results indicate that droplet diameter and associated mass are more determinant of turfgrass canopy penetration than droplet velocity. Absorption of ^{14}C was up to four times greater when radioactive glufosinate and glyphosate were exposed to zoysiagrass stolons compared to leaves. Zoysiagrass leaves treated with ^{14}C-glufosinate had more rapid ^{14}C absorption than those treated with ^{14}C-glyphosate. More ^{14}C translocated out of the treated area following ^{14}C-glyphosate treatment compared to that of ^{14}C-glufosinate, and both herbicides moved more readily from stolon to leaves than from leaves to stolon. Avoiding induction-type nozzles and raising spray height may slightly decrease penetration of droplets into a zoysiagrass subcanopy, but 28 to 45 % of droplets still reached the middle and lower canopy layers in this research. Thus, subcanopy green leaves and stems will likely be exposed and will readily absorb both glyphosate and glufosinate. These data suggest that limiting green tissue at application may be a more prudent approach to safening semidormant zoysiagrass from nonselective herbicides when compared to altering sprayer configuration.

Nomenclature: ‘Meyer’ zoysiagrass, *Zoysa japonica* Steud.; XR nozzles, extended range Teejet flat fan nozzles Teejet 11006 nozzle; TTI, Turbo Teejet Induction 11006 nozzles.

Keywords: Zoysiagrass, canopy penetration, spray droplets, spray penetration, absorption and translocation, nozzles, boom height, pressure, stolons, stems.

Introduction

Winter-dormant zoysiagrass (*Zoysia* spp.) is commonly characterized by brown foliage with no green tissue protruding the canopy. However, it has been noted that close inspection of dormant zoysiagrass can reveal green tissue at the base of stems and subcanopy leaves that are at least partially green (Velsor et al., 1989). Nonselective herbicides, such as glyphosate or glufosinate, are commonly used to control winter weeds in dormant turfgrass, but only four peer-reviewed papers have reported dormant-zoysiagrass response to these herbicides (Hoyle and Reeves, 2017; Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013). These four papers indicate that glyphosate and/or glufosinate did not injure zoysiagrass when treated “prior to green-up” or “during dormancy.” Aside from an ancillary mention of subcanopy green tissue observed by Velsor et al. (1989), the researchers made no mention of canopy characteristics such as number of green leaves or stolons. In three cases, glyphosate and/or glufosinate injured zoysiagrass when applied later in the season to partially-green zoysiagrass canopies (Rimi et al., 2012; Velsor et al., 1989; Xiong et al., 2013). Thus, past work clearly indicates that partially-green zoysiagrass canopies may be severely injured by nonselective herbicides and suggests that at some unreported level of canopy dormancy, herbicide injury is no longer problematic.

Despite evidence of glyphosate and glufosinate being safely applied to dormant zoysiagrass in a few scientific papers and product labels for these herbicides indicating their use on “dormant turfgrass”, there is considerable fear within the turfgrass industry regarding using these herbicides on dormant zoysiagrass. For example, extension bulletins from Arkansas and Tennessee recommend against treating dormant zoysiagrass with glyphosate (Boyd, 2016; Brosnan and Breeden, 2011). The senior author’s personal experience as an extension specialist in Virginia involves numerous turfgrass managers reporting that glyphosate inconsistently injured zoysiagrass when applied to turf that had been characterized as “fully dormant.” The two most logical explanations for inconsistent injury following dormant sprays is 1) the practitioners all mistook partially green turf as dormant, or 2) subcanopy green leaves and stems were sometimes exposed to spray droplets and were able to absorb the herbicide. In this paper, we explore some factors that may contribute to variable spray exposure to subcanopy turf layers and the extent to which subcanopy stems may absorb glyphosate or glufosinate when compared to leaves.

It has been suggested nozzles that produce coarse to very coarse droplets could increase turf canopy penetration (Shepard et al., 2006). Although several studies have evaluated the influence of nozzle type and application volume on surface coverage of turf and associated weed or disease control (Benelli, 2016; Ferguson et al., 2016; Fidanza et al., 2009; Kaminski and Fidanza, 2009; McDonald et al., 2006; Neal et al., 1990; Vincelli and Dixon, 2007), factors that govern spray penetration into turf canopies have not been reported. A recent thesis from the University of Tennessee examined exposure of stem, sheath, and leaves in a zoysiagrass canopy and found subcanopy stem exposure to increase with increasing application volume (Benelli, 2016) but the associated paper has not yet been published. Previous research examing canopy penetration across a range of spray parameters and nozzle types has been conducted primarily in cropping systems studying weed control. The results from these studies have found varying results with respect to droplet diameter and canopy penetration (Derksen et al., 2008; Hanna et al., 2009; Knoche, 1994; Wolf and Daggupati, 2009; Zhu et al., 2004). Wolf and Daggupati (2009) observed improved penetration into a dense soybean canopy from fine and medium droplets. Zhu et al. (2004) found that air induction nozzles produced more spray deposition into the bottom of soybean canopies, while conventional flat fan nozzles had the lowest spray penetration. Derksen et al. (2008) also observed better soybean canopy penetration with medium and coarse droplets compared to fine droplets. In contrast, Hanna et al. (2009) observed no difference between droplet sizes with respect to soybean canopy penetration.

Spray droplet size and velocity affect the structure of spray deposits and the droplet potential to drift (Lake, 1977). Research has shown that in general larger droplets correspond with higher droplet velocities (Nuyttens et al., 2007). Creech et al. (2015) determined nozzle design and application pressure caused the most significant changes to spray droplet size. Increasing spray pressure reduces droplet size and may increase associated spray coverage (Liao et al., 2020). As the distance between spray nozzles and the targeted pest or "boom height" increases, spray deposition to the target decreases due to drift (Balsari et al., 2007) and droplet velocities, especially of smaller droplets, also decrease (Goering et al., 1972). The most common type of nozzles used in turfgrass settings is the flat fan nozzle (Shepard et al., 2006). Air induction nozzles were designed to produce larger droplets than standard flat fan nozzles at a given pressure for drift control (Etheridge et al., 1999). It is conceivable that manipulating nozzle type, pressure, or boom height could alter the penetration of droplets into a turf canopy due to the aforementioned effects these parameters have on droplet size and speed.

Assuming herbicide can be delivered to subcanopy layers of zoysiagrass turf, stem exposure becomes more relevant. The ability of the herbicides glyphosate and glufosinate to absorb into zoysiagrass stems has not been previously reported. In fact, no information could be found relating herbicidal absorption into turfgrass stems. Glyphosate and glufosinate can be absorbed by roots, stems, and leaves (Pline et al., 2001; Steckel et al., 1997; Thomas et al., 2004; Wills, 1978; You and Barker, 2002), so stem absorption into zoysiagrass is plausible. Considering the paucity of data regarding spray penetration into subcanopy layers of zoysiagrass turf and stem absorption of commonly used herbicides during zoysiagrass dormancy, studies were conducted in to investigate these factors. The objectives of this research are 1) determine the effect of nozzle type, nozzle height above the target, and spray pressure on droplet size and velocity and the penetration of spray droplets into a zoysiagrass turf canopy using a colorant tracer assessed via fluid extraction and spectrophotometric analysis and 2) evaluate absorption and translocation of ^{14}C-glyphosate and ^{14}C-glufosinate in ‘Meyer’ zoysiagrass when applied to leaves or subtending stolons.

Materials and Methods

Spray Penetration Study

A study was conducted at the Virginia Tech Glade Road Research Facility in Blacksburg, VA (37.233250, -80.435983), in the spring of 2019 and repeated in the spring of 2020. ‘Meyer’ zoysiagrass plugs (10.8-cm diameter by 15-cm deep) were collected using a commercial golf course cup cutter (Lever Action Hole Cutter, Pair Aide Products, Lino Lakes, MN) from a field site maintained at a regular mowing height of 5.7 cm. The site’s soil type was a Duffield silt loam (fine-loamy, mixed, active, mesic, Ultic Hapludalfs)-Ernest silt loam (fine-loamy, mixed, superactive, mesic Aquic Fragiudults) complex, with a pH of 6.8 and 4.8 % organic matter. The intact turf plugs were placed into 14-cm-diameter pots and backfilled with native soil to ensure the turf canopy was level with the top of the pot. The experiment was set up as a completely random design with eight replications. Treatments were arranged as a two (nozzle) by two (pressure) by two (height) factorial.

Spray nozzles included XR Teejet 11006 (XR) and Turbo Teejet Induction 11006 (TTI) flat spray tips (TeeJet Technologies, Springfield, IL). Nozzles were chosen based on their ability to produce different droplet sizes and velocities and to represent common nozzles used in the turf industry (Shepard et al., 2006). These two nozzles were operated at all possible combinations of 103 and 414 kPa pressures and 25 and 61-cm heights above the turf canopy. A colorant (Bullseye Spray Pattern Indicator, Milliken Chemical, Spartanburg, SC) was mixed 50 % by volume with water and the application volume was held constant at 374 L/ha^{-1} for all treatments by manipulating boom speed. Spray was allowed to dry for 24 hours at room temperature and the turf canopy of each treated plug was then dissected with scissors using reference jigs to guide the cutting operation. The 5.7-cm canopy was separated into three approximate 1.9-cm layers. Canopy leaf and stem material was suspended in 50-ml water and shaken for 10 seconds. The suspension were filtered with Whatman No. 42 filter paper (Whatman International Ltd., Maidstone, England) using a Buchner funnel under negative pressure. Another 10-ml of water was used to rinse the extraction container, Buchner funnel, and filter paper. Preliminary studies indicated a 97 ± 2 % extraction efficiency. The resulting extract was further diluted 1:4 with deionized water and a 2-ml aliquot was added to a 5-ml cuvette and absorbance was measured via spectrophotometer (Genesys 5, Thermo Spectronics, Rochester, NY) at 650 nm. Colorant concentration was calculated based on a standard curve comprised of 30 known dye concentrations between 0 and 5000 ppm measured for absorbance at 650 nm in triplicate.

After colorant extraction, the remaining plant material was dried for 48 h at 60 °C and weighed on a 10,000th g scale. The material was then combusted at 500 °C for 5 h and the resulting ash weight was subtracted from the dried plant material weight to exclude any possible soil or other contaminants. A nontreated comparison was subjected to the same process and served as background for the spectrophotometric analysis. In addition, all plant material incised from each of the three canopy layers of nontreated plugs were subjected to surface area measurement (LI-

3100 Area Meter, Li-Cor, Lincoln, NE) to determine the approximate relationship between biomass and surface area (Figure 1). The resulting slope and intercept were used to relate biomass to estimated surface area so that extracted colorant could be expressed as ml colorant m^{-2} tissue surface area.

Data were subjected to a combined analysis of variance with sums of squares partitioned to reflect the effects of year, replication, nozzle type, pressure, and spray height above the turf canopy. Year was considered a random variable and the mean square of all main effects or interactions of nozzle, pressure, and height were tested with the mean square associated with their interaction with year (McIntosh, 1983). Data were pooled over year only if year interactions were insignificant at $\alpha = 0.05$. Significant effects or interactions were subjected to Fisher's Protected LSD test at $\alpha = 0.05$.

Droplet Diameter and Velocity

The same nozzles, pressures, and heights of the previous study were duplicated using the same spray equipment mounted to a custom-built droplet analyzer. Droplet characteristics for each of the eight combinations of nozzle, pressure, and height were measured via high-speed video analysis at 15,000 frames per second. A high-speed video camera (Edgertronic, San Jose, CA) was positioned with a field of view spanning 23 mm in height with the upper edge of the viewable area set to 25 and 61-cm below the operating nozzles. A 700 watt halogen light bank illuminated the viewing area. Imaged droplets were restricted to a 2-cm field of view via a 2 cm by 40 cm opening in a plastic shield positioned between the spray nozzle and the camera viewing area. Scale objects were used to determine on-screen size relationships and the video background consisted of repeating black and white bars of known width for additional reference. Droplet diameters were measured manually from the output display and converted to actual size via scale relationships.

Droplet velocities were determined by counting the number of video frames required for a given droplet to traverse the 23 mm vertical viewing area and subjecting the data to the following equation.

$$Y = ((1/(f/15000))*\text{Ɣ})/1000 \qquad \text{Eq. 1}$$

Where Y is the droplet speed in m s^{-1}, f is the observed number of frames required for vertical traversal, and Ɣ is the vertical viewing area of the camera in mm. Droplets that appeared unusually slow compared to the majority were assumed to have impinged the depth-of-field restriction opening and were excluded from analysis. Horizontal diameter and velocity were determined for 100 randomly chosen droplets for each level of nozzle, pressure, and height. Data were subjected to analysis of variance with sums of squares partitioned to reflect the effects of year, rep, nozzle type, pressure, and height above the droplet assessment area. Mean square test were conducted as mentioned previously. Significant effects or interactions were then subjected to linear or 2nd order polynomial regression analysis to relate droplet diameter to droplet velocity.

Absorption and Translocation Study

‘Meyer’ zoysiagrass plant material was collected from the Glade Road Research Facility in Blacksburg, VA (37.233250, -80.435983), in September of 2020. Plugs of zoysiagrass were collected using a commercial golf course cup cutter (Lever Action Hole Cutter, Pair Aide Products, Lino Lakes, MN) (10.8 cm diameter by 15-cm in depth). Once removed from the field, they were placed into the greenhouse and fertilized with Miracle-Gro All-purpose Plant Food 24-18-6 (The Scotts Miracle-Gro Company, Marysville, OH). Greenhouse conditions consisted of 27 C ± 6 C with approximately 420 µmol m^{-2} s^{-1} of photosynthetically active radiation in the greenhouse and irrigated every 24 hours in order to maintain active growth. After acclimating to greenhouse conditions, plugs were dissected, and 6-cm grass sprigs that contained at least three green leaves and a 1 cm long subtending stolon were collected. Three sprigs were placed into 9- cm diameter glass petri dishes with wet germination paper (76# Heavy Weight Seed Germination Paper Circles, AnchorSeed Solution, St. Paul, MN). Prior to sprigs being placed into petri-dishes, they were dipped into a 0.59 mg ai/L solution of fluxapyroxad + pyraclostrobin (Lexicon Intrinsic fungicide, BASF Corporation, Research Triangle Park, NC) to prevent large patch (*Rhizoctonia solani*) (Amaradasa et al., 2014). The germination paper was watered by syringe as needed to prevent desiccation with a ¼ strength Hoagland's Modified Basal Salt solution (pH 6.5) (MP Biomedicals, Solon, OH) to maintain adequate moisture. Petri-dishes with associated glass covers were placed into a growth chamber set to 30/25 °C day/night temperature with approximately 330 μmol m^{-2} s^{-1} of photosynthetically active radiation.

The study was arranged in a completely randomized design with four replications and repeated in time for a total of eight replications. Treatments were arranged in a split-plot design with a two herbicide by two application-placement factorial arrangement of main plots and three levels of harvest time as subplots. Each three-leaf shoot with subtending stolon was considered an experimental unit and each petri dish comprised a main plot. After two days in the growth chamber, three 1-µl droplets containing 4.0 kBq ^{14}C-glyphosate or ^{14}C-glufosinate were spotted on the leaf or stolon. The glyphosate (phosphonomethyl-14C, specific activity = 50 µCi/mmol, purity 99 %) spotting solution was converted to the isopropylamine salt by combining 200 µl ^{14}C glyphosate acid with 1.6 µl isopropyl amine, then adding 0.8 % v/v MON56164 (Monsanto Company) (nonionic polyethoxylated tallow amine) surfactant. Radiolabeled glufosinate (glufosinate hydrochloride, specific activity = 51.8 µCi/mg) spotting solution contained a 0.1 % v/v nonionic surfactant (Induce, Helena Chemical Company, Collierville, TN).

Shoots with subtending stolons were randomly assigned within petri dishes to be harvested at 4, 24, and 72 hours after treatment. At harvest, the treated leaf or stolon was removed from the plant and vortexed for 30 seconds in 10 ml of methanol: deionized water (1:1) plus 0.25 % v/v nonionic surfactant to remove unabsorbed herbicide. A 0.5-ml aliquot of the resulting rinsate was added to 15 ml of scintillation fluid (Sciniti Verse LC Cocktail Scitanalyzed, Fisher Scientific, Pittsburgh, PA) and analyzed with a liquid scintillation spectrometer (LSS) (6500 Multipurpose Scintillation Counter, Beckman Coulter Inc, Indianapolis, IN) to determine total unabsorbed radioactivity. Following the rinse, the treated portion and remaining shoot or stolon were then immersed into liquid nitrogen, followed by grinding of plant material with a mortar and pestle. Two ml of extraction solution (4:1 methanol: deionized water) was added to the pulverized plant tissue and homogenized using the motor and pestle. The ground plant material and extraction solution were suction filtered using a Buchner funnel fitted with Whatman No.1 filter paper (Whatman International Ltd., Maidstond, England). The mortar, pestle, funnel, and filter paper were further rinsed with an additional 8 ml of extraction solution. A 0.5-ml aliquot from the resulting extract was then added to 15 ml of scintillation fluid, and total absorbed radioactivity was determined by LSS as described previously.

Since the “harvest-time” subplots had variance structure consistent with repeated measures, trends in recovered radioactivity over time were subjected to linear regression by replicate and resulting slopes were subjected to ANOVA as described previously. Recovered radioactivity at the 3-d harvest was analyzed likewise. The effects of herbicide, placement, and herbicide by placement were tested using the mean square associated with their interaction with trial (McIntosh, 1983). Data were pooled over insignificant interactions for the purposes of mean separation. Appropriate means were separated, as described previously.

Results and Discussion

Spray Penetration Study

The interaction of year or pressure with nozzle, height, or their interaction was insignificant ($P > 0.05$) and data were pooled to present the significant nozzle type by height interaction ($P < 0.05$) for percentage recovered colorant at each canopy level (Table 1). It should be noted that the average recovery based on the targeted colorant application rate was 69 % ± 14 % (data not shown). Visual inspection of the thatch layer at the soil surface suggested that a large percentage of colorant was delivered to the soil surface. We assume variable delivery to the soil may have contributed to the aforementioned recovery rate. When expressed as a percentage of recovered colorant, the majority of colorant was recovered from the upper canopy and recovery from the middle and lower canopy levels exhibited an apparent stepwise reduction in all cases, although these comparisons were not statistically analyzed due to spatial variance structure. In the top canopy layer, the XR nozzle at 61-cm height captured 73 % of recovered colorant and more than all other treatments (Table 1). A concomitant decrease in recovered colorant was noted from this nozzle and height combination in the middle (17 %) and lower (11 %) canopies when compared to either nozzle at the 25-cm height and to TTI nozzles at the 61-cm height. The data suggest that TTI nozzles at either height and XR nozzles at the 25-cm height deposited 56 to 59 % of droplets in the upper canopy, 24 to 27 % of droplets in the middle canopy, and 17 to 19 % of droplets in the lower canopy. These data agree with those of Zhu et al. (2004) in that induction type nozzles penetrate crop canopies more than XR nozzles. Researchers have demonstrated that small droplets less than 150 µm are prone to drift and reduce subcanopy deposition to crops when applied at greater distances between nozzle and target (Yates et al., 1985). These studies were often conducted under sustained or generated wind while we avoided influence of wind by choosing a wind-free day and actively avoiding spray during any detectable gusts.

Similar trends to percentage recovery data were noted when extracted colorant was expressed as quantity m^{-2} of plant tissue (Table 1) estimated based on the relationship of biomass to tissue surface area (Figure 1). Again, the nozzle type by height interaction was the highest-ranked interaction with a significant $P < 0.05$ (data not shown). And interactions based on year or pressure were insignificant ($P > 0.05$). When using the XR nozzle, 13-ml of colorant was deposited m^{-2} to the upper canopy tissue from a distance of 61-cm compared to 11-ml of colorant deposited from a distance of 25-cm. Similar reductions in colorant were also noted in middle and lower canopy levels when the XR nozzle was operated at 61-cm from the turf. Also similar to percentage recovery data, the TTI nozzles deposited equivalent amounts of colorant regardless of height above the turf. These data clearly show that substantial exposure to subcanopy tissue will occur regardless of nozzle type, pressure, or height between nozzle and turf when application volume is at 374 L ha^{-1}. Previous work has shown that application volume can increase efficacy of fungicides when targeting subcanopy pathogens (Benelli, 2016). To impart better safety to zoysiagrass turf when using nonselective herbicides during dormancy, avoiding induction nozzles and raising boom height could be coupled with reduced application volume. Further research is required to test this theory.

Droplet Diameter and Velocity

The ANOVA of droplet velocities indicated that the three-way interaction of nozzle by pressure by height was significant ($P = 0.0373$) but further analysis by nozzle showed that TTI nozzles were not significantly influenced by height with respect to droplet velocity ($P > 0.05$). In Figure 2, the relationship of droplet diameter to droplet velocity is shown as 2nd order polynomial regressions comprising all observations across both heights for TTI nozzles and the interaction of pressure by height for XR nozzles (Figure 2). Since these data are comprised of hundreds of randomly selected droplets, they may partially suggest the distribution of droplet diameters. However, the purpose of the data in Figure 2 was to determine droplet velocity and a more rigorous random sampling was conducted to calculate volume median diameter by nozzle and pressure. These are shown as shaded vertical bars on Figure 2 but were calculated based on over 100 droplets per assessment from randomly chosen screenshots of the high-speed video.

In all cases, droplet velocity increased with increasing droplet diameter but these trends were dependent on the nozzle type, pressure, and in the case of XR nozzles, the height above the target (Figure 2). TTI nozzles exhibited a range of measurable droplet sizes between 90 and 1620 µm regardless of pressure (Figure 1). Increasing pressure did, however, increase droplet velocity from TTI nozzles by about 2.5 to 5.0 m s^{-1} for droplets ranging from 400 to 1620 µm. In contrast, the range of droplet diameters for XR nozzles was strongly influenced by pressure being 60 to 900 µm at 103 kPa and 60 to 540 µm at 414 kPa. Increasing pressure from 103 kPa to 414 kPa nearly doubled droplet velocity from XR nozzles at droplet diameters greater than 200 µm. Unlike TTI nozzles, height above the target had a substantial effect on droplet velocity from XR nozzles at either pressure with droplets > 200 µm increasing by 2 to 4 m s^{-1}. These data agree with measured droplet velocities reported by Goering et al. (1972). The change in droplet speed as distance from target increases has also been calculated and measured (Goering et al., 1972).

The fact that distance from the target influenced droplet velocity similarly between TTI and XR nozzles but only XR nozzles exhibited a difference in deposition at various zoysiagrass canopy layers (Table 1), suggests that droplet diameter and associated mass may have more influence on canopy penetration than velocity. The lack of difference in canopy penetration by TTI nozzles at different observed droplet velocities may also be influenced by the air-entrained design of droplets generated by induction nozzles. These droplets are designed to splatter upon impact (Grayson et al., 1991) and could result in a more uniform distribution in lower turfgrass canopies regardless of droplet speed. Despite slight differences in lower canopy penetration that we have attributed partially to greater distance between XR nozzles and the turf, the fact remains that all canopy layers are exposed to a large percentage of droplets regardless of nozzle, pressure, or height. These data suggest that underlying stems can be exposed at any level of zoysiagrass dormancy.

Herbicide absorption and translocation in stems vs. leaves

The interaction of trial with herbicide, placement, or herbicide x placement was not significant ($P > 0.05$) for any response variable. Data were pooled over trial to examine the significant herbicide x placement interaction for both the slope of responses over harvest times and observed values of recovered radioactivity at 3 d after treatment from rinsing the treated area, extracting from shoot tissue, and extracting from stolon tissue ($P = 0.0005$ to 0.0044) (Table 2). Recovered radioactivity from the rinse of treated leaves declined 6.6 % d^{-1} for glyphosate and less than glufosinate, which had a radioactive recovery that declined 12 % d^{-1}. Radioactivity recovered from the rinse of treated stolons did not vary between herbicides with respect to temporal trends (Table 2). When applied to leaves, glufosinate resulted in nearly double the daily increase of recovered radioactivity from shoots compared to glyphosate, indicating more rapid foliar absorption. Glufosinate had a three-fold slower rate of recovered radioactivity accumulation in shoots when applied to stolons compared to when applied to leaves (Table 2). Although glyphosate accumulated half the recovered radioactivity of glufosinate, daily radioactivity accumulation by glyphosate was not dependent on placement to leaf or stolon. Radioactivity accumulation d^{-1} in stolons was dependent on where the herbicides were initially placed but not on herbicide. Placement to leaves did not result in more than 0.3 % d^{-1} of recovered radioactivity moving to the subtending stolon, while treatment to stolons led to 3.9 to 5.1 % d^{-1} of recovered radioactivity moving upwards to the shoots.

At 3 d after treatment, 75 % of applied radioactivity associated with glyphosate was still on the treated leaf compared to only 44 % remaining on leaves following glufosinate treatment. When applied to stolons, only 20 % of applied radioactivity associated with glufosinate remained on the stolon surface after 3 d (Table 2). Glyphosate did not differ with respect to placement to leaf or stolon in the amount of recovered radioactivity found in shoots after 3 d. Glufosinate, had more radioactivity in shoots following leaf treatment than following stolon treatment. Neither herbicide led to appreciable radioactivity accumulation in stolons after 3 d when applied to leaves but 14 to 24 % of recovered radioactivity was measured in shoots following treatment to stolons. Surprisingly, total absorption of radioactivity 3 d following application to stolons was approximately 80 % and more than when applied to leaves (25 to 56 %). No examination of herbicide absorption into turfgrass stems has previously been reported. These data suggest that herbicides applied to dormant zoysiagrass have potential to absorb into subcanopy stolons. Most nozzle, pressure, and height configurations tested in the canopy penetration study indicated that as much as 19 % of applied droplets will be deposited to the lower canopy (Table 1). We did not account for dead sheath material that would shield a large percentage of subcanopy zoysiagrass stems as these were removed to reduce error associated with the absorption study. Assuming that 30 % of subcanopy stems are not covered by sheath material, a reasonable estimate of herbicidal exposure to subcanopy stems is approximately 6 % of the target rate. If 80 % of this material is absorbed, then the rate load in dormant zoysiagrass would be approximately 4.8 % of the targeted herbicide rate. As green leaves are added to the canopy, the risk of injury by herbicides will increase exponentially as has been observed when glyphosate or glufosinate are applied to partially green zoysiagrass canopies. Absorption of radioactivity following treatment of either herbicide to stolons was rapid as evidenced by scanning the 4-h harvest data (data not shown) or by multiplying slopes in Table 2 by three (number of days to 3-d harvest) then adding the value to the observed values for 3-d after treatment in Table 2. For example, glyphosate applied to stolons had a slope of rinse radioactivity of -9.8, which yields -29.4 % and added to the observed value at the 3-d harvest of 20 % equals 49.4 %. The actual mean for radioactivity in the rinse at 4 h after glyphosate treatment was 50.4 % (data not shown). Thus, about half of the radioactivity had absorbed into stolons within 4 hr. These results indicate that irrigation would not be an effective means of reducing injury from direct spray deposition. The best way to safen zoysiagrass from direct spray deposition is to limit the percentage of green tissue or exposed stems in the canopy and reduce the amount of spray that reaches lower canopy levels. Our data suggest that increasing spray height and avoiding induction-type nozzles may help in that regard.

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Table 1. Influence of nozzle type and height above zoysiagrass turf on total colorant and colorant per tissue surface area[a] extracted from three positions within the turf canopy. Data were averaged over two years (2019 and 2020) and two spray pressures (103 and 414 kPa).

	Spray boom	Total colorant by canopy position			Colorant per leaf/stem surface area by canopy position		
Nozzle type	height	Top	Middle	Bottom	Top	Middle	Bottom
	(cm)	——— (% of recovered) ———			——— (ml m^{-2}) ———		
Flat Fan	25	59	24	17	11	5.8	3.4
	61	73*	17*	11*	13*	4.0*	1.9*
LSD (0.05)		6.5	4.0	3.7	NS	1.6	1.2
Turbo Teejet Induction	25	56	27	18	13	6.0	3.6
	61	56*	25*	19*	10*	5.9*	3.3*
LSD (0.05)		NS	NS	NS	NS	NS	NS

[a] Tissue surface area was estimated via area meter for all excised portions of nontreated turf canopies and subjected to linear regression to determine its relationship to biomass (see Figure 1). The resulting slope and intercept were used to estimate surface area based on biomass of all tissue that had been subjected to colorant extraction.

Means followed by a * were significantly different between nozzle type within a given spray boom height.

Figure 1. Relationship between surface area and biomass of excised leaf and stem material from nontreated turf.

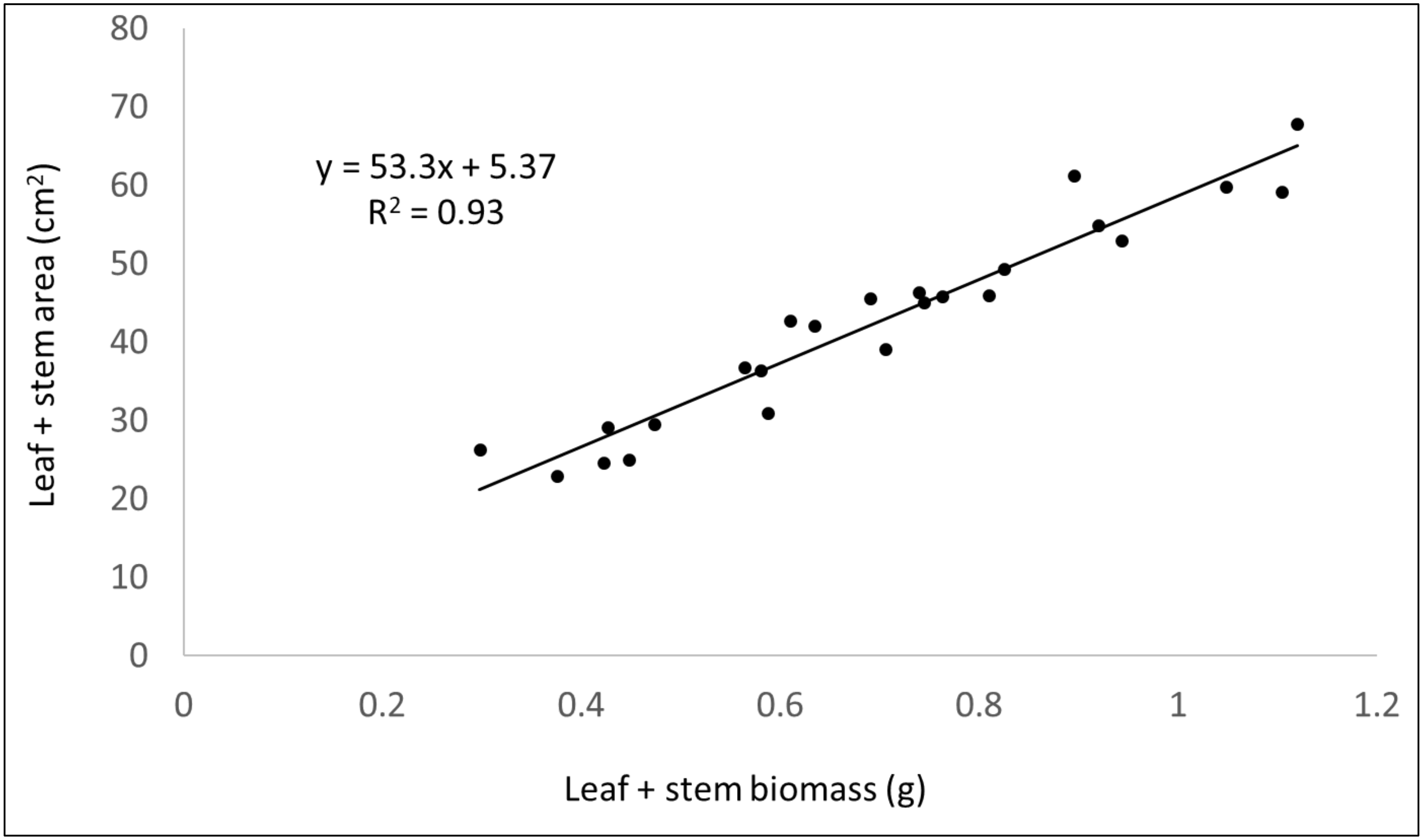

Figure 2. Relationship between horizontal droplet diameter and droplet velocity from Turbo Teejet Induction and XR Teejet flat spray tips each at 103 and 414 kPa pressure and XR Teejet also separated by boom height. Droplets were measured via high-speed video at 15,000 frames s^{-1}. The Turbo Teejet Induction data are pooled over 25 and 61 cm boom heights.

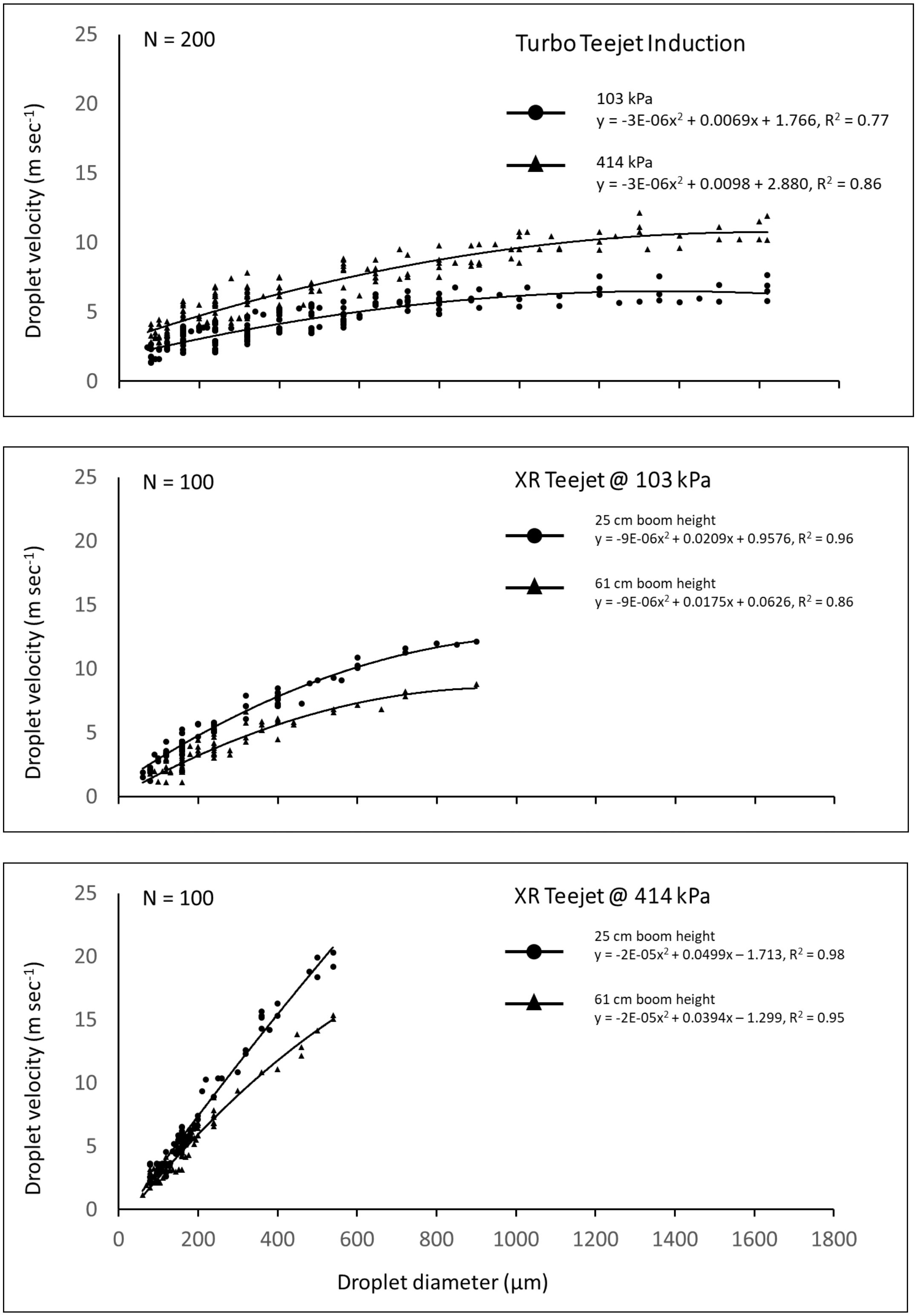

Table 2. Influence of herbicide and application placement on slope of recovered ^{14}C radioactivity x time in days and extracted radioactivity at 3 d after application from a rinse of the treated portion (rinse), extraction from shoot tissue (shoot), and extraction from stolon tissue (stolon) for zoysiagrass sprigs that each included a 1-cm stolon subtending a 3 cm leaf shoot.

		Recovered radioactivity x time (d)			Recovered radioactivity at 3 d after treatment		
Nozzle type	Placement	Rinse	Shoot	Stolon	Rinse	Shoot	Stolon
		—— (% of recovered d^{-1}) ——			—— (% of recovered) ——		
Glyphosate	leaf	-6.6*	6.2*	0.3	75*	24*	1
	stolon	-9.8	5.1	4.7	20	24	56
LSD (0.05)		NS	NS	4.0	13	NS	8.4
Glufosinate	leaf	-12*	12*	0.2	44*	54*	2
	stolon	-11	3.9	6.9	22	14	64
LSD (0.05)		NS	7.3	3.3	20	19	11

[a] Shoots were treated on the adaxial surface of the newest, fully expanded leaf and stolons were treated on the on the adaxial surface of the internode preceding the shoot.

Means followed by a * are significantly different between herbicides within a given placement location (leaf versus stolon) at $\alpha = 0.05$.