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Effect of temperature and heat units on zoysiagrass response to herbicides during post-dormancy transition

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Abstract

In the transition zone, turfgrass managers generally utilize the dormancy period of warm-season turfgrass to apply herbicides for managing winter annual weeds. Although this weed control strategy is common in bermudagrass [*Cynodon dactylon* (L.) Pers.], it has been less adopted in zoysiagrass (*Zoysia* spp.) due to variable turfgrass injury during post-dormancy transition. Previous research reported that air temperature could affect weed control and crop safety from herbicides. Growth-chamber studies were conducted to evaluate zoysiagrass response to glyphosate and glufosinate as influenced by three different temperature regimes during and after treatment. A field research study was conducted at four site-years to assess the influence of variable heat-unit accumulation on zoysiagrass response to seven herbicides. In the growth-chamber study, glufosinate injured zoysiagrass more than glyphosate and reduced time to reach 50% green cover reduction, regardless of the rate, when incubated for 7 d under different temperature levels. When green zoysiagrass sprigs were incubated for 7 d at 10 C, the rate of green cover reduction was slowed for both herbicides; however, green cover was rapidly reduced under 27 C. After treated zoysiagrass plugs having 5% green cover were incubated at 10 C for 14 d, glyphosate-treated plugs reached 50% green cover in 22 d, similar to nontreated plugs but less than the 70 d required for glufosinate-treated plugs. Zoysiagrass response to glyphosate was temperature dependent, but glufosinate injured zoysiagrass unacceptably regardless of temperature regime. Diquat, flumioxazin, glufosinate, and metsulfuron + rimsulfuron injured zoysiagrass at 200 or 300 growing-degree days at base 5 C (GDD_{5C}) application timings, but foramsulfuron and oxadiazon did not injure zoysiagrass regardless of GDD_{5C}. The relationship of leaf density to green turf cover is dependent on zoysiagrass mowing height, and both metrics are reduced by injurious herbicides. Research indicates that glufosinate injures zoysiagrass more than glyphosate, and the speed and magnitude of herbicide injury generally increase with temperature.

Introduction

Turfgrass managers often apply nonselective herbicides such as glyphosate to control winter annual weeds in dormant zoysiagrass (*Zoysia* spp.) (Johnson 1980; Toler et al. 2007; Xiong et al. 2013). Cold temperatures and shorter daylengths induce dormancy in zoysiagrass, and dormant turf is generally more tolerant of herbicides (Baltensperger 1962; Beard 1972; Hendry et al. 1987). Nonselective herbicides are more commonly utilized on dormant turf in the climatic transition zone or areas that represent the northern extent of the zoysiagrass growing region in the United States (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 regarding the safety of herbicide use on zoysiagrass during winter. Furthermore, the concept of "dormancy" in zoysiagrass is poorly understood and remains unaddressed in the scientific literature (Patton et al. 2017). Zoysiagrass dormancy is associated with the "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 glufosinate or glyphosate is of great concern to turfgrass managers, as these herbicides can substantially delay the development of green turfgrass in spring and reduce zoysiagrass quality (Rimi et al. 2012; Xiong et al. 2013). Glufosinate and glyphosate reduced 'Meyer' zoysiagrass (*Zoysia japonica* Steud.) quality equivalently when applied later in the spring season to partially green turfgrass (Xiong et al. 2013). Previous research that 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) indicated that these herbicides are safe to apply on "dormant zoysiagrass" or "prior to green-up," but only 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 'Zeon' zoysiagrass [*Zoysia matrella* (L.) Merr.] (Rimi et al. 2012).

Patton and Reicher (2007) documented that different species of zoysiagrass are strongly responsive to temperature. The variable response to cold temperatures was reported to be the determining factor for the geographic distribution of zoysiagrass species, with *Z. japonica* being more adapted to colder regions than *Z. matrella*, which is more prevalent in 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 turfgrass managers are planning to apply herbicides to control winter annual weeds, with temperature variations 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 (Derr and Serensits 2016; Kudsk and Kristensen 1992).

Temperature affects herbicide efficacy by influencing plant growth and development (Kudsk and Kristensen 1992). Temperature alterations can cause physiological changes in the plant, resulting in differential rates of herbicide absorption and translocation (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 conditions (Derr and Serensits 2016; Kudsk and Kristensen1992). Jordan (1977) indicated that increasing temperatures from 22 to 32 C significantly increased glyphosate activity on bermudagrass [*Cynodon dactylon* (L.) Pers.]. 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 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 preliminary observations and those of Velsor et al. (1989) indicated that some level of green tissue would be present when most zoysiagrass is treated with herbicides in winter in the transition zone. We hypothesized that green zoysiagrass shoots or zoysiagrass canopies with small amounts of green leaf tissue will respond differently to glyphosate and glufosinate under different temperature regimes maintained 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 temperature or heat units on zoysiagrass response to herbicides during post-dormancy transition had not been previously conducted. Therefore, the objectives of this research were to (1) determine zoysiagrass response to glyphosate and glufosinate applications as influenced by three different temperature regimes maintained during and after treatment, (2) determine the impact of extended periods (more than 7 d) of three temperatures during or after nonselective herbicide applications on zoysiagrass during the post-dormancy transition, and (3) determine the influence of variable heat-unit accumulation on in-field zoysiagrass growth parameters and injury response to herbicides.

Materials and Methods

Zoysiagrass Response to Glyphosate and Glufosinate under Short-Term Exposure to Different Temperature Regimes

A growth-chamber experiment was conducted at the Virginia Tech Glade Road Research Facility in Blacksburg, VA (37.23°N, 80.44° W) in the spring of 2018. The study was implemented as a randomized complete block design, split-plot arrangement with three temperature levels as the main plot and a factorial arrangement of two herbicides applied at three rates as subplots. The study comprised six temporal blocks with one replication each and five subsample sprigs per experimental unit. Semidormant 'Meyer' *Z. japonica* plugs of 10-cm diameter and 10-cm height were collected on March 6, 2018, from a field site mown regularly to a height of 6.35 cm. After plugs were collected from the field, intact plugs were acclimated in a greenhouse at 27 ± 6 C for 48 h under 420 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) with a 13-h photoperiod and irrigated every 24 h. 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, Research Triangle Park, NC) to prevent disease and placed in 8.9-cm-diameter petri dishes on moistened blotter paper disks (76# heavy weight seed germination paper, Anchor Paper, St Paul, MN) (Amaradasa et al. 2014). Petri dishes were 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. Temperature regimes were selected to represent the range of temperatures that occur during the post-dormancy transition of zoysiagrass in Virginia. Similarly, light-intensity level for growth chambers was also selected to represent the daily light integral experienced by turfgrass during post-dormancy transition in Virginia. Temperatures throughout the experiment in the growth chambers and greenhouse were monitored using external data loggers equipped with temperature sensors (using HOBO U12 4-channel outdoor external data loggers and TMCE 20-HD air/water/soil temperature sensors, Onset, Bourne, MA) programmed to record temperatures at 15-min intervals. Sprigs were allowed to acclimate at respective temperatures for 12 h and were then removed from petri dishes; sprayed over the top with glyphosate (Roundup Pro® Concentrate, Bayer Environmental Sciences, Research Triangle Park, NC) at 130, 260, or 520 g ae ha^{-1} or glufosinate (Finale®, Bayer Environmental Sciences) at 420, 840, or 1,680 g ai ha^{-1}; and returned to growth chambers. A nontreated check was included for each temperature regime.

Herbicide treatments were applied with a CO_2-pressurized spray chamber calibrated to deliver 280 L ha^{-1} using an XR

8003E even flat spray tip (TeeJet® Technologies, Wheaton, IL). For the duration of the experiment, water was applied directly to the blotter paper disk to moisten sprigs, 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 at 3 d after treatment (DAT). Petri dishes were moved to the greenhouse at 7 DAT and incubated for an additional 14 d. The greenhouse temperature was maintained at 27 C ± 6 C, and supplemental light provided 420 μmol m^{-2} s^{-1} PAR for a 13-h photoperiod each day.

Green cover of zoysiagrass sprigs was assessed by collecting aerial digital images of each petri dish at 0, 2, 4, 7, 10, 14, 18, and 21 DAT. The digital images, taken at a height of 40 cm, 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 DAT, the treated sprigs were oven-dried at 70 C for 48 h and weighed for biomass. Detected green pixels of treated sprigs were converted to a percent reduction in green cover relative to nontreated sprigs for each temperature by replicate combination. Percent reduction was subjected to sigmoidal nonlinear regression (Equation 1) to explain the relationship of time after treatment.

$$C = ae^{be^{kT}} \qquad [1]$$

where C is the percentage green cover, a is the upper asymptote for the final green cover, b and k are estimated parameters, e is the base of the natural logarithm, and T is the 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 (Equation 2)

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

where GCR_{50} is the time in days to reach a 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 using PROC GLM in SAS v. 9.2 (SAS Institute, Cary, NC). Sums of squares were separated to account for temperature, replication, replication by temperature, herbicide, and temperature by herbicide as appropriate for the split-plot design (McIntosh 1983). Mean squares associated with main effects or interactions containing herbicide were tested for residual error. Mean squares of the main plot temperature were tested by replication by temperature (McIntosh 1983). Appropriate means were separated with Fisher's protected LSD ($P \leq 0.05$).

Zoysiagrass Response to Glufosinate and Glyphosate under Long-Term Exposure to Different Temperature Regimes

A separate experiment was initiated in the spring of 2019 and repeated in the spring of 2020 to evaluate the effect of extended periods of temperatures surrounding glyphosate and glufosinate applications to zoysiagrass. The experiment was implemented as a randomized complete block design with four temporal replications in each of the two runs. Treatments were arranged in a split-plot design with three temperature regimes as the main plot and three treatments as subplot. Semidormant Meyer *Z. japonica* plugs of 10-cm diameter and 10-cm height were removed from the same location as plugs used in the first growth-chamber experiment. Once the zoysiagrass plugs were removed from the field, the intact plugs were placed into 14-cm-diameter pots back-filled with native soil. After the plugs were potted, they were oversprayed with fluxapyroxad + pyraclostrobin (Lexicon Intrinsic® fungicide, BASF) at 644 g ai ha^{-1} to prevent disease development. Turfgrass plugs were then placed in the greenhouse for 48 h under conditions similar to those in the previous experiment to initiate post-dormancy transition. 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 PAR at 330 μmol m^{-2} s^{-1}. After 12 h, plugs with 5% visual green cover were removed from the growth chambers and sprayed over the top with glyphosate at 520 g ae ha^{-1} or glufosinate at 1,680 g ai ha^{-1} and returned to same chambers. A nontreated control was included for each temperature regime. Herbicide treatments were applied using the same spray chamber as the previous study. No irrigation was applied until 3 d after herbicide treatment to ensure herbicides had adequate time to be absorbed. Subsequent irrigation was applied over the top of plugs as needed while in growth chambers. The treatments were maintained under the same temperature regimes used in the short-term study but for a longer (14 DAT) duration. Plugs were moved to the greenhouse at 14 DAT and incubated for an additional 42 d. Greenhouse conditions were maintained as previously discussed.

Zoysiagrass green cover was assessed via aerial images and digital image analysis similar to that previously described, with the exception that whole zoysiagrass green canopies were assessed rather than just three sprigs. Spectral reflectance data were collected using a handheld, hyperspectral field radiometer (PSR-1100F, Spectral Evolution, Haverhill, 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 reference calibration panels. Two subsamples of reflectance data were collected per plug and averaged. The reflectance data were 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). NDVI data of turfgrass canopies have been found to correlate with green cover and turfgrass quality (Carrow et al. 2010). Data were collected at 0, 2, 4, 7, 10, 14, 21, 28, 42, and 56 DAT.

Unlike the sprig study, in which 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, data were not converted 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 with 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} (Equation 2).

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

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

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 per day by dividing by the number of days spanned by the assessment period. Campbell and Madden (1990) 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 in which long durations are assessed by repeated measures. NDVI AUPC per day data were separated and analyzed as NDVI in-chamber AUPC per day for plugs and NDVI post-chamber AUPC per day for plugs to examine the possible differences in turf recovery once removed from the growth chambers. The GTC_{50} and NDVI AUPC per day data were subjected to ANOVA using PROC GLM, with sums of squares separated to account for year, temperature, replication, replication by temperature (year), herbicide, temperature by herbicide, year by herbicide, and year by temperature by 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 by temperature with year nested (McIntosh 1983). Appropriate means were separated with Fisher's protected LSD ($P \leq 0.05$).

Effect of Heat Units on Zoysiagrass Response to Herbicides

A field research study was conducted at two unique sites in each of two spring seasons (2016 and 2017) at the Virginia Tech Turfgrass Research Center in Blacksburg, VA (37.21°N, 80.41°W). Each year, one of the sites contained a mixed stand of 'Companion' *Z. japonica* and 'Zenith' *Z. japonica* zoysiagrass mown with a rotary mower to a height of 3.8 cm, and the second site consisted of Meyer zoysiagrass mown with a reel mower to a height of 1.8 cm. The soil at both trial sites was a Groseclose urban land complex (fine, mixed, semiactive, mesic, Typic Hapludults) 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 the active growth period, 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 factorial arrangement of two application timings and seven herbicides, replicated three times. Plots were 1.8 m by 1.8 m. Diquat, flumioxazin, foramsulfuron, glufosinate, glyphosate, metsulfuron + rimsulfuron, and oxadiazon at 560 g ai ha^{-1}, 428 g ai ha^{-1}, 28.9 g ai ha^{-1}, 1,680 g ai ha^{-1}, 520 g ae ha^{-1}, 21 g ai ha^{-1} + 17.5 g ai ha^{-1}, and 3,383 g ai ha^{-1}, respectively, were evaluated during the experiment. Treatments were applied with a CO_2-pressurized backpack sprayer equipped with four Turbo TeeJet® Induction 11004 spray tips (TeeJet® Technologies, Springfield, IL) calibrated to deliver 280 L ha^{-1}. GDD_{5C} (growing-degree day at base 5 C) accumulation started January 1 each year, as calculated in other studies (Patton et al. 2004; Rimi et al. 2012; Schiavon et al. 2011; Severmutlu et al. 2011). Initial herbicide applications for the early timing took place 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. Both of these timings would be considered later than recommended timing 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). Because zoysiagrass has been injured by herbicides when treated at later spring timings (Velsor et al. 1989; Xiong et al. 2007), reportedly after the post-dormancy transition, these timings were chosen to represent "early green-up" and "mid–green-up."

Zoysiagrass percentage green cover, turfgrass injury, green leaves per square decimeter, and NDVI data were collected at 0, 7, 14, 21, 28, 42, and 56 DAT. Zoysiagrass injury was assessed visually on a 0% to 100% scale, where 0% indicates that plots had equivalent green zoysiagrass vegetation compared with the nontreated and 100% indicates all green vegetation of the zoysiagrass was eliminated. An injury of 30% or greater was considered unacceptable. Zoysiagrass green cover was assessed as a visually estimated percentage of the plot area. Zoysiagrass green leaf counts were collected by counting all green or partially green leaves present within a 10 cm by 10 cm randomly chosen location in each plot. Measurements of NDVI were collected using a Crop Circle ACS 210 multispectral analyzer (Holland Scientific, Lincoln, NE) affixed 43 cm above the turf that collected 50 ± 5 readings per plot that represented a 0.5 m by 1.6 m area of turf canopy in the center of each plot.

Zoysiagrass percentage green cover, turfgrass injury, green leaves per square decimeter, and NDVI data over time were converted to the AUPC using the same formula and parameters described previously. Zoysiagrass injury maxima were recorded as the maximum value observed at any assessment data. Zoysiagrass NDVI, green cover, and green leaves per square decimeter 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 per day, allow for estimating trends over time that otherwise would not be evident from AUPC per day data. Slope and AUPC per day data for zoysiagrass green cover, NDVI, and green leaves per square decimeter, along with injury maxima, were subjected to ANOVA using PROC GLM in SAS v. 9.2 with sums of squares partitioned to reflect replication, site, year, and site by year as random effects, while herbicide, application timing, and herbicide by application timing were considered 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 are discussed separately by site, year, or site by year if significant interaction was detected ($P < 0.05$). Otherwise, data were pooled over site and 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 per square decimeter was further investigated via linear regression.

Results and Discussion

Zoysiagrass Response to Glyphosate and Glufosinate under Short-Term Exposure to Different Temperature Regimes

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 green 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 more than 80% green cover reduction within 5 DAT, while the colder temperature of 10 C only caused 5% or less green cover reduction of zoysiagrass (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 sprigs being moved to the warmer greenhouse conditions of 27 C at 7 DAT (Figures 1 and 2).

The interaction of temperature by 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 reach GCR_{50} more rapidly than glyphosate, regardless of temperature

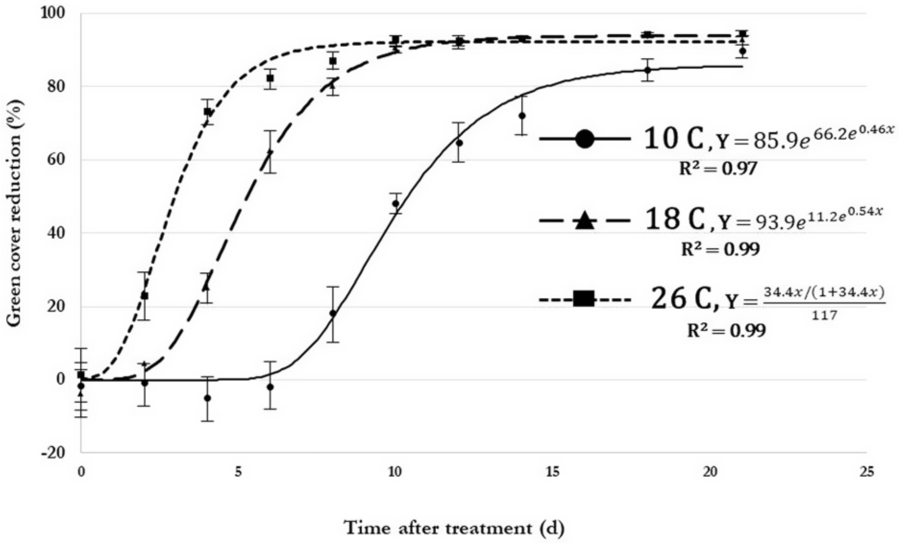

Figure 1. Average effect of three glyphosate rates on green cover reduction of zoysiagrass sprigs relative to nontreated sprigs over time as influenced by exposure to three constant temperature regimes for the first 7 d after treatment (DAT). All sprigs were exposed to 27 C starting at 7 DAT.

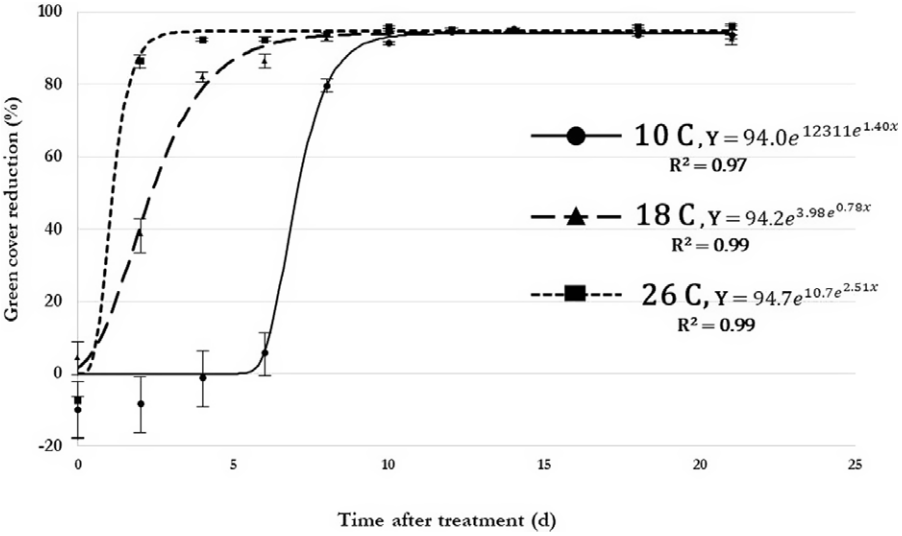

Figure 2. Average effect of three glufosinate rates on green cover reduction of zoysiagrass sprigs relative to the nontreated over time as influenced by exposure to three constant temperature regimes for the first 7 d after treatment (DAT). All sprigs were exposed to 27 C starting 7 DAT.

regime (Table 1). The 7-d incubation period of zoysiagrass sprigs 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 (Table 1). The difference between the number of days required for zoysiagrass sprigs to reach GCR_{50} between the 10 and 27 C temperatures is 6.3 and 7.1 d for glyphosate and glufosinate, respectively (Table 1). Because 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 increase accordingly.

Glufosinate has been shown to injure plants more quickly than glyphosate (Bromilow et al. 1993; Steckel et al. 1997). Plants typically exhibit herbicide symptoms in 3 to 5 d after glufosinate treatment (Pline et al. 1999a; Steckel et al. 1997) and 4 to 7 d after glyphosate treatment (Pline et al. 1999b). The rapid activity of glufosinate results from inhibiting glutamine synthetase and causing a buildup of ammonia in plant cells (Pline et al. 1999a; Wendler et al. 1990). Our findings were similar to those of Anderson et al. (1993), who reported that glufosinate injury to *Setaria viridis* 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 with 27 C (Table 1) is further evidence that temperature caused 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).

Zoysiagrass Response to Glyphosate and Glufosinate under Long-Term Exposure to Different Temperature Regimes

To better evaluate the apparent differences between zoysiagrass response to herbicides in controlled temperature chambers

Table 1. Influence of constant temperature regimes incubation for 7 d on the digitally assessed green cover and biomass of zoysiagrass sprigs after herbicide treatment.[a]

Treatment	Time to GCR$_{50}$[b]			Biomass		
	10 C	18 C	27 C	10 C	18 C	27 C
		d			g	
Nontreated	—	—	—	0.0945a	0.1115a	0.1192a
Glyphosate	10a	5.1b	2.7c	0.0449a	0.0377a	0.0400a
Glufosinate	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

[a] Means within a given level of herbicide and across the three temperature levels 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.

[b] GCR$_{50}$, green cover reduction of zoysiagrass by 50%.

compared with the greenhouse, the study involving zoysiagrass plugs explored whole-canopy responses after a greater (14-d) temperature incubation period, and the NDVI per day data were separated by the time spent in the constant-temperature growth chambers and the subsequent "post-chamber" time spent in the greenhouse (Table 2). The interaction of herbicide by temperature was significant for NDVI in-chamber AUPC per day ($P = 0.0186$) and NDVI post-chamber AUPC per day ($P = 0.0038$), but neither in-chamber ($P = 0.0523$) nor post-chamber ($P = 0.4155$) interactions were dependent on year.

The in-chamber NDVI AUPC per day for nontreated, glyphosate-treated, and glufosinate-treated turf plugs was statistically similar when zoysiagrass was exposed to 10 C (Table 2). However, the NDVI AUPC per day for in-chamber zoysiagrass differed when temperatures increased to 18 or 27 C (Table 2). At both of these higher temperatures, glufosinate lowered NDVI per day compared with glyphosate or the nontreated (Table 2). While plants were in the temperature chambers, nontreated zoysiagrass exhibited a stepwise increase in NDVI per day as temperature increased (Table 2). 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 short- and long-term temperature exposure studies indicate that glufosinate may be more injurious to zoysiagrass than glyphosate based on the speed of activity (Tables 1 and 2). To better explore this possibility, one can examine the post-chamber NDVI per day 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 under 27 C.

Zoysiagrass turf did not vary in post-chamber NDVI per day based on the different temperatures to which it had previously been exposed for any level of herbicide treatment (Table 2). Thus, any lasting effects of the 14-d temperature treatment before moving plants to the greenhouse did not alter NDVI per day during the 42-d post-chamber incubation. This suggests that changes in NDVI per day during the post-chamber period must have been solely due to herbicide. For all temperatures, glufosinate reduced zoysiagrass turf NDVI per day more than glyphosate and the nontreated during the post-chamber period (Table 2). These data suggest that zoysiagrass was either injured less or recovered more following glyphosate treatment when compared with glufosinate treatment. Differential zoysiagrass injury between glufosinate and glyphosate has not been previously reported, but these differences may explain why glufosinate has never been labeled for use in dormant zoysiagrass in contrast to several glyphosate products that are labeled.

In the long-term temperature exposure study, 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 reached 50% green cover in 22 d compared with 70 d for plugs treated with glufosinate (Table 2). These data suggest that the cold temperature over a 14-d duration may have reduced zoysiagrass injury from glyphosate treatment but not from 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 the data collected on zoysiagrass sprigs from the short-term exposure study (Table 1). Posttreatment incubation of zoysiagrass at both higher temperatures led to herbicide-induced delays in time to reach 50% green cover (Table 2). 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 to 62 d (Table 2).

Effect of Heat Units on Zoysiagrass Response to Herbicides

Injury maxima following seven unique herbicide treatments exhibited a significant herbicide by application timing interaction ($P = 0.0336$) but were not dependent on site ($P = 0.0645$), year ($P = 0.4603$), or site by year ($P = 0.0529$). Maximum injury on zoysiagrass 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 3). Glufosinate injured zoysiagrass turf more than all other herbicides at both application timings (Table 3). 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 3). All other treatments would be deemed too injurious to use at application timings of ~200 GDD$_{5C}$ or later (Table 3). This increased zoysiagrass injury by glufosinate compared with glyphosate is supported by results on both sprigs and turf-canopy plug experiments in controlled-temperature conditions (Tables 1 and 2). 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 were more similar to our early application timing, where we observed substantially more injury from glufosinate compared with glyphosate.

The interaction of herbicide by application timing was significant for zoysiagrass turf injury AUPC per day ($P = 0.0015$) and not

Table 2. Influence of long-term incubation period under different temperature levels on zoysiagrass normalized difference vegetation index (NDVI) and time to reach 50% green turf cover of zoysiagrass plugs after herbicide treatment.[a,b]

		NDVI in-chamber AUPC			NDVI post-chamber AUPC			GTC_{50}		
Treatment	Rate[c]	10 C	18 C	27 C	10 C	18 C	27 C	10 C	18 C	27 C
	g ai/ae ha^{-1}		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	1,680	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

[a] Abbreviations: AUPC, area under progress curve; GTC_{50}, time to reach 50% green turf cover of zoysiagrass.

[b] Means within a given level of herbicide and across the three temperature levels 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.

[c] Glyphosate rate was based on g ae ha^{-1}, while glufosinate rate was in g ai ha^{-1}.

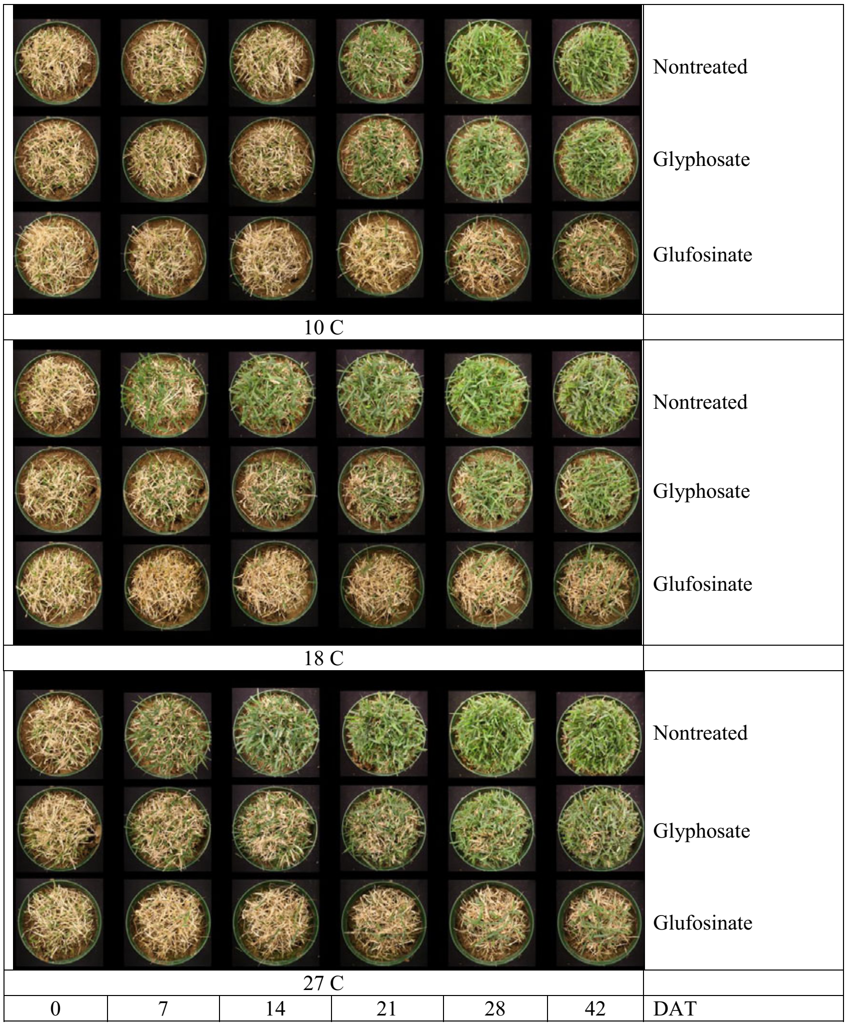

Figure 3. One of eight replicates showing a selection of assessment dates between 0 and 42 d after treatment (DAT) to demonstrate the effects of the extended incubation period of different temperature regimes on zoysiagrass green turf cover over time when 10-cm turf plugs were treated with herbicides at 5% green cover.

dependent on site (P = 0.0742), year (p = 0.0507), or site by year (P = 0.2224). Average injury per day was higher at the ~300 GDD_{5C} timing than the ~200 GDD_{5C} timing for all herbicides except flumioxazin (Table 3). Glyphosate applied at ~200 GDD_{5C} caused a temporal trend of 8.3% injury d^{-1} and was equivalent to the safest herbicides evaluated (Table 3). At ~300 GDD_{5C}, however, glyphosate was much more injurious and averaged 36% injury d^{-1} (Table 3). As was observed for injury maxima (Table 3), injury per day caused by glufosinate on zoysiagrass was significantly greater than for all other herbicides, regardless of application timing. These data suggest that, although applications beyond 100 GDD_{5C} should be discouraged, glyphosate may be applied more safely over a broader application period than glufosinate.

Table 3. Influence of herbicide and application timing (200 ± 60 and 300 ± 30 GDD$_{5C}$) on percentage maximum zoysiagrass injury and both slope over time and area under the progress curve (AUPC) per day of normalized difference vegetation index (NDVI), percentage green turf cover, and number of green leaves per square decimeter.[a,b,c]

		Injury maxima		Injury AUPC		NDVI × time		NDVI AUPC	
Treatment	Rate[d]	200 GDD$_{5C}$	300 GDD$_{5C}$	200 GDD$_{5C}$	300 GDD$_{5C}$	200 GDD$_{5C}$	300 GDD$_{5C}$	200 GDD$_{5C}$	300 GDD$_{5C}$
	g ai/ae ha^{-1}	%		avg. % d^{-1}		Δ 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	1,680	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 (%) × time		Green cover AUPC		Green leaf dm^{-2} × time		Green leaf dm^{-2} AUPC	
		200 GDD$_{5C}$	300 GDD$_{5C}$	200 GDD$_{5C}$	300 GDD$_{5C}$	200 GDD$_{5C}$	300 GDD$_{5C}$	200 GDD$_{5C}$	300 GDD$_{5C}$
		Δ d^{-1}		avg. % d^{-1}		Δ d^{-1}		avg. no. 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	1,680	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

[a] Abbreviations: Δ d^{-1}, change in response per day; avg. % d^{-1}, average % per day; avg. no. d^{-1}, average number per day; GDD$_{5C}$, growing-degree day at base 5 C.

[b] Average daily values were based on AUPC following seven assessments over a 56-d period after treatment to semidormant zoysiagrass, averaged over four site-years.

[c] Means followed by an asterisk (*) were significantly different between herbicide application timings. Means followed by a dagger (†) were significantly different compared with the nontreated based on single degree-of-freedom comparisons.

[d] Glyphosate rate is based on g ae ha^{-1}, while other herbicides were applied based on g ai ha^{-1}.

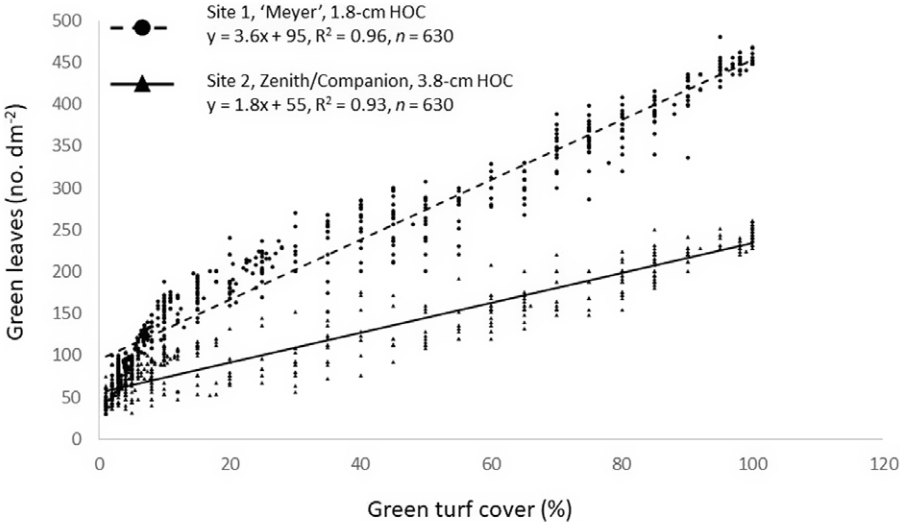

Figure 4. Relationship between visually estimated zoysiagrass green cover and green leaves per square decimeter from studies conducted over 2 yr at two sites spatially separated by 0.1 km and characterized by different zoysiagrass varieties and mowing heights of cut (HOC).

The interaction of herbicide by application timing for NDVI slopes over time and average NDVI AUPC per day were significant ($P < 0.05$) and not dependent on site, year, or site by year ($P > 0.05$). Slopes of NDVI over time indicate that nontreated turf gained 0.0085 NDVI per day, consistent with an increase in zoysiagrass quality (Table 3). Thus, the average daily NDVI-based AUPC of 0.5 would have taken approximately 59 d to achieve based on the slope and assuming an intercept of zero (Table 3). 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. NDVI temporal slopes were significantly greater at the earlier application timing for flumioxazin, glufosinate, glyphosate, and metsulfuron + rimsulfuron, likely due to increased injury by the abovementioned herbicides at the later application timing (Table 3). The average NDVI AUPC per day also differed between application timing for glufosinate and glyphosate (Table 3) and was also in agreement with injury data. Generally, the more injurious herbicide treatments significantly reduced the average NDVI AUPC per day compared with the nontreated (Table 3).

Like other response variables, the interaction of herbicide by application timing was significant for green turf cover slopes over time and green turf cover AUPC per day ($P < 0.05$). 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 3). 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 3).

The interaction of herbicide by application timing was also significant for green leaves per square decimeter slopes over time and green leaves per square decimeter AUPC per day ($P < 0.05$) and not dependent on site, year, or site by year ($P > 0.05$). A strong site main effect for green leaves per square meter slope over time with $P < 0.0001$ and $F = 4{,}434$ suggests that the magnitude of green leaf accumulation over time was influenced by site, but lack of significant herbicide and timing interactions with site indicate these variables impacted green leaves per square meter 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 per square meter is shown in Figure 4. Linear trend lines explain at least 93% of data variance and indicate that Meyer zoysiagrass maintained at 1.8 cm gains 3.6 new leaves for each 1% 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 with the fairway-height turf (1.8 cm). 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 (Figure 4). Interestingly, these data prove that zoysiagrass can have between 25 to more than 50 green leaves dm^{-2} in the canopy before any surface green cover being observed. These data support the previous report of subcanopy green leaves in the turf that was considered "dormant" (Velsor et al. 1989). They 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 per square decimeter over time indicates that glufosinate lowers green leaves per square decimeter regardless of application time (Table 3). Glyphosate and metsulfuron + rimsulfuron reduce the rate of green leaf accumulation only when applied at the later application timing (Table 3). Lack of differences between herbicides that were evident in some other response variables but not evident in the slope of green leaves per square decimeter could be attributed 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 per square decimeter 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 were confounded with related effects on stunting of new leaf production to create variability in green leaf per square decimeter response to herbicides and application time. This issue also affected the average green leaves per day based on AUPC (Table 3). These data allowed us to distinguish differences in green leaves per square decimeter per day only for the most injurious herbicide, glufosinate (Table 3).

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 evaluated. 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 assessed herbicides except glufosinate and metsulfuron + rimsulfuron. When treated at approximately 300 GDD_{5C}, only foramsulfuron and oxadiazon can safely be used for zoysiagrass if no delay in development occurs. 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 documented 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 per square decimeter 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. Future work will assess whether heat-unit relationships to green leaf counts, zoysiagrass green canopy cover, and zoysiagrass response to herbicides is consistent over a broad region of the U.S. Southeast compared with Blacksburg, VA.

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