Integrated Strategies for Controlling Warm-Season Turfgrass Weeds

A Thesis

Presented for the Master of Science

Degree

The University of Tennessee, Knoxville

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December 2011

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DEDICATION

To my parents, Donna and Jim for their unwavering love and support throughout the years. I would not be where I am today without them.

To my brother, Brian.

To the love of my life, Karen McInnis.

ACKNOWLEDGEMENTS

For their support and guidance throughout my time at the University of Tennessee, I thank my committee members: Dr. Brandon Horvath, Dr. Dean Kopsell, and Dr. Tom Mueller. I give special thanks to my advisor, Dr. Jim Brosnan. Through countless hours of mentorship, he taught me how to be the scientist I am. His energy and encouragement serve as constant sources of inspiration. I do not know where I would be without him.

I thank Mr. Greg Breeden for his assistance and expertise on numerous aspects of my projects, saving many hours of my time. For their countless hours of assistance with my experiments, I thank Shane Breeden, Daniel Farnsworth, Jake Huffer, Andrew Reis and Veronica Sublett. I thank the ETREC farm crew for their assistance in plot maintenance.

Thank you my fellow graduate students, Jesse Benelli, Lucas Freshour, Will Haselbauer, Matt Hollan, Pat Jones, David Shell and Adam Thoms for their camaraderie, friendship and advice. You all really made my time in Tennessee a fun one. Together we have had many good times hanging out, crushing buffets and beers.

Thank you Mr. Matt Naedel, Mr. Jeff Borger and Dr. Pete Landschoot for your mentorship and allowing me to realize my passion for research as an undergraduate at Penn State.

ABSTRACT

Herbicidal inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) such as mesotrione, topramezone and tembotrione were evaluated in greenhouse experiments for activity against bermudagrass. While topramezone and tembotrione exhibited greater activity than mesotrione, none of these herbicides provided acceptable bermudagrass control. These herbicides reduced leaf tissue chlorophyll and carotenoid pigment concentrations in bermudagrass. Changes in turfgrass pigmentation were quantified using HPLC analogy as well as evaluations of visual bleaching and measurements of chlorophyll fluorescence yield (F_v/F_m). Results indicated that these more expeditious methods of evaluating HPPD-inhibiting herbicide activity (visual evaluations and F_v/F_m) cannot be used to successfully predict turfgrass pigmentation following applications.

Mesotrione and topramezone are efficacious against small (< 2-tiller) smooth crabgrass plants. Field studies were conducted in 2010 and 2011 to evaluate efficacy of mesotrione and topramezone on 3- to 5-tiller smooth crabgrass in combination with different rates of nitrogen fertilizer. Greenhouse experiments were conducted to evaluate the dose-response of smooth crabgrass to mesotrione and topramezone with nitrogen fertilizer. Increased mesotrione and topramezone efficacy was observed with added nitrogen fertilizer in all experiments. Greenhouse experiments indicate that nitrogen fertilizer reduces the amount of mesotrione and topramezone required to control smooth crabgrass. Further research indicated that this enhancement was due to increased activity in the shoot meristem which could be caused by increased translocation of the herbicide.

Dallisgrass (*Paspalum dilatatum*), bermudagrass (*Cynodon* spp.) and smooth crabgrass (*Digitaria ischaemum*) are problematic weeds in turfgrass throughout the

southeastern United States. Infestations negatively affect the aesthetic and functional quality of desirable turfgrass stands. Additionally, these species compete with turfgrasses for light, water, and nutrients. Therefore, selective control of these species is warranted.

The herbicide fluazifop-p-butyl is commonly applied to control dallisgrass in tall fescue (*Festuca arundinacea*); however, control is often short-lived. A two-year field study was conducted to evaluate the efficacy of several herbicide combinations with fluazifop-p-butyl for dallisgrass control when applied at different growing degree-day-(GDD) based application timings. Results of this research indicate that application timing significantly impacts long-term dallisgrass control with fluazifop-p-butyl.

Results of experiments presented here indicate simple strategies can be used to influence the efficacy of herbicides against problematic turfgrass weeds.

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LITERATURE REVIEW

DALLISGRASS BIOLOGY

Dallisgrass (*Paspalum dilatatum* Poir.) is a C_4 grass belonging to the plant Family Poaceae, Subfamily Panicoideae and Tribe Paniceae is a warm season, perennial grassy weed native to Northern Argentina, Uruguay and Southern Brazil (Beard 2002; Holt 1956). Introduced to the United States in 1879, it is adapted to the warmer, humid climates of the mid-Atlantic and southeast (Beard 2002). Un-adapted to arid climates, dallisgrass survival west of eastern Texas is limited (Holt 1956; Piper 1922, 1924).

The coarse leaf texture and bunch type growth habit of dallisgrass differentiates it from several desirable turfgrasses used throughout the transition zone. Dallisgrass infestations reduce the aesthetic and functional quality of the turfgrass sward (Figure 1.1). Its bunch-type growth habit and increased vertical growth rate (relative to desirable turfgrass) disrupt surface uniformity, creating an injury hazard on athletic fields (Elmore and Cudney 2001). Objectionable dallisgrass seedheads are also present throughout much of the growing season as the long flowering culm often escapes the mowing reel (Elmore and Cudney 2001). Seed production, likely through apomixis, is the main mechanism of dallisgrass dispersal (Bashaw and Holt 1958).

Tolerance to traffic and high soil moisture enhance dallisgrass persistence in turfgrass stands. In a dallisgrass population previously adapted to flooding, Rubio et al. (1995) reported greater biomass after 60 days of flooding, compared to plants maintained at field capacity for soil moisture. Similarly, Mollard et al. (2008) reported an increase in photosynthesis for dallisgrass plants subjected to 60 days of flooding. Moreover, Striker et al. (2006) reported dallisgrass biomass was not reduced under simulated cattle traffic following flooding conditions. Evaluating the response of dallisgrass along a soil

moisture gradient, Henry et al. (2008a) found dallisgrass was better able to compete with hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *Cynodon transvaalensis* Burtt Davy) as soil moisture increased.

Though close mowing and/or cultural practices and conditions promoting growth of desirable turfgrass reduce dallisgrass infestation, once established, it has shown the ability to survive in a variety of turfgrass environments (Elmore and Cudney 2001; Henry et al. 2009). This adaptation, combined with its ubiquity in the southeastern United States, leaves chemical control as the most effective option for large-scale dallisgrass removal.

OPTIONS FOR DALLISGRASS CONTROL IN TURF

Chemical methods of dallisgrass control, or suppression in turfgrass are limited to the organic arsenical, sulfonylurea (SU), arlyoxyphenoxy propionate (AOPP) and triketone herbicide classes.

The organic arsenical monosodium methanearsonate (MSMA) is registered for grassy weed control use in warm-season turf (Anonymous, 2010). While its mode of action is unknown, MSMA causes foliar necrosis in susceptible plants (Senseman 2007). Single, or sequential applications of MSMA at 2.5 kg ai/ha are reported to provide dallisgrass suppression (Henry et al. 2007). Pre-treatment with MSMA can increase the amount of dallisgrass control provided by SU herbicides. Henry et al. (2007) reported two sequential applications of the SU herbicide foramsulfuron at 0.15 kg/ha provided 60% dallisgrass control 1 month after initial treatment (MAIT), but only 5% control 1 year after initial treatment (YAIT). Comparatively, pre-treatment with MSMA at 2.5

kg/ha 2 weeks prior to a single application of foramsulfuron improved control to 85% and 29% 1 MAIT and 1 YAIT, respectively (Henry et al. 2007). Increased control was at least partially attributed to an increase in absorption and translocation of foramsulfuron following pre-treatment with MSMA (Henry et al. 2008b). EPA restrictions currently prohibit sale of MSMA for athletic field and residential turf use and forthcoming restrictions will prohibit use on sod farms and golf courses after 2013 (United States EPA, 2009). Thus, future dallisgrass control programs will be based on the use of SU, AOPP, and HPPD-inhibiting herbicides alone or in mixtures with one another.

SU herbicides inhibit acetolactate synthase (ALS) thereby inhibiting production of branched chain amino acids (LaRossa and Schloss 1984). In addition to foramsulfuron, suppression of dallisgrass with sequential applications of SU herbicides trifloxysulfuron-sodium and nicosulfuron has also been observed (Strahan et al. 2005; Henry and Yelverton 2005; Ricker et al. 2005); however, these herbicides do not provide long-term dallisgrass control.

The triketone herbicide mesotrione inhibits HPPD, indirectly inhibiting carotenoid production (Senseman 2007). Mesotrione exhibits efficacy against broadleaf and grassy weeds in several cool-season turfgrasses (Beam et al. 2006; Borger et al. 2009; Jones and Christians 2005; McElroy et al. 2007; Willis et al. 2006). Reicher et al. (2005) reported mesotrione activity on dallisgrass with control up to 3 MAIT following sequential applications at 0.19, and 0.28 kg ha^{-1}.

AOPP herbicides inhibit acetyl-CoA carboxylase, the enzyme catalyzing the conversion of malonyl-CoA to acetyl-CoA, the first step in fatty acid synthesis (Burton et al. 1989). Inhibition of phospholipid production in susceptible grasses results in cell

membrane failure, particularly in meristematic regions where formation of new cells occurs (Senseman 2007). Registered for use in tall fescue, a single application of the AOPP herbicide fluazifop-p-butyl (hereafter referred to as fluazifop) at 0.105 kg/ha was reported to provide 90% dallisgrass control 76 days after treatment in early spring with minimal tall fescue injury (Anonymous, 2009b; Brosnan et al. 2010a). Evers et al. (2002) also demonstrated dallisgrass desiccation in a pasture with fluazifop at rates ≥ 0.14 kg/ha, but saw almost no control 1 YAIT. Brosnan et al. (2010a) suggested the seasonal variability in fluazifop efficacy may be related to environmentally influenced plant development.

GROWING DEGREE DAY MODELING

Changes in plant development have been linked to environmental conditions for over 250 years. In 1735, René A. F. Réaumur published the first work relating the summation of mean daily air temperatures to plant development. This summation became known as Réaumur's thermal constant of phenology (Wang 1960). Since this introduction, versions of Réaumur's constant have been modified for use with specific crops. In 1750, Adanson excluded all temperatures below 0 ºC, while Gasparin chose to exclude all temperatures below 5 ºC (Wang 1960). Variables accounting for the influence of other abiotic factors such as daylength, water and nutrient availability, light wavelength and light quantity have been considered (Masle et al. 1989; McMaster and Wilhelm 1997; Nuttonson 1948; Wang 1960). Today, growing degree day (GDD) accumulation is commonly used to quantify the influence of air temperature on plant development throughout a growing season. The GDD accumulation is commonly

calculated by the formula GDD = $[(T_{max} - T_{min})/2] - T_{base}$, where T_{max} is the daily maximum air temperature, T_{min} is the daily minimum air temperature and T_{base} is the lowest temperature at which the biological process of interest (e.g., plant growth) does not advance (McMaster and Wilhem 1997). Using this equation, GDD values greater than zero are summed throughout the growing season to form an "accumulated" value.

Since its introduction, the GDD equation has been used to build models useful in predicting biological phenomena. In winter wheat (*Triticum aestivum* L.), GDD models with various base temperatures can accurately predict phenological events such as heading, kerneling and maturity better than other methods, such as calendar date (McMaster and Smika 1988). In forage systems, GDD accumulation can estimate plant growth stage, as well as the grazing readiness and quality of various cool-season grass species (Frank and Hofmann 1989; Hill et al. 1995; Mitchell et al. 2001). In turfgrass, GDD accumulation can determine annual bluegrass (*Poa annua*) and Kentucky bluegrass (*Poa pratensis* L.) seedhead emergence, smooth crabgrass (*Digitaria ischaemum* (Schreb.) ex Muhl.) emergence, Kentucky bluegrass root formation and viable creeping bentgrass (*Agrostis stolonifera* L.) root length (Danneberger and Vargas 1984; Fidanza et al. 1996; Koski et al. 1988; Schlossberg and Karnok 2002). Accurately determining the occurrence of these phenological events allows turfgrass managers to maximize efficacy of annual bluegrass seedhead suppressants, as well as preemergence crabgrass control products and postemergence broadleaf herbicide applications (Branham and Danneberger 1989; Fidanza et al. 1996; Scheicher et al. 1995).

GDD modeling can optimize postemergence herbicide application timing. Schleicher et al. (1995) evaluated the use of a GDD model with a base temperature of 50

°F (GDD_{50F}) to maximize efficacy of ester and amine formulations 2,4-D + 2,4-DP against dandelion (*Taraxacum officinale* F.H. Wigg.). Applied before 130 to 145 GDD_{50F} 2,4-D ester resulted in 57% control, but control improved to >80% when the herbicide was applied after 130 to 145 GDD_{50F}. Applications of 2,4-D + 2,4-DP amine and 2,4-D + 2,4-D ester responded similarly. In a separate study conducted in a growth chamber, Schleicher and Throssell (1996) reported 43% and 87% control of dandelion with 2,4-D + 2,4-DP ester applied at 110 GDD_{50F} and 160 GDD_{50F}, respectively. Increased control was concomitant with greater translocation of a phloem mobile tracer (with properties similar to 2,4-D) at 160 GDD_{50F} compared to 110 GDD_{50F} (Schleicher and Throssell 1996). In the only investigation of dallisgrass control involving GDD based application timing, Brosnan et al. (2010a) reported >90% control of dallisgrass 76 DAIT from single and sequential applications of fluazifop made in early spring (<160 GDD_{10C}). When applied in early summer (>500 GDD_{10C}) control was <40% 76 DAIT. Given the seasonal variability in dallisgrass control with fluazifop, a more complete investigation to determine the optimum GDD based application timing is warranted.

OVERSEEDING

Cultural practices promoting healthy turfgrass help eliminate voids in the canopy (Turgeon 2005). Eliminating these voids with desirable turfgrass species reduces niches for weed sustenance (Watschke and Engel 1994). Effects of overseeding on weed control in turfgrass are varied. Overseeding with perennial grasses controlled Canada thistle (*Cirsium arvense* (L.) Scop) and downy brome, (*Bromus tectorum* L.) in pastures (Whitson and Koch 1998; Wilson and Kachman 1999). Experiments conducted on

football pitches and golf course fairways found overseeding + verticutting either increased or had no effect on weed populations (Larsen et al. 2004; Larsen and Fischer 2005). Elford et al. (2008) found perennial ryegrass overseeding did not affect weed populations on in-use municipal athletic fields. However, Kopec and Gilbert (1999) found that, perennial ryegrass overseeding in combination with sulfentrazone resulted in less annual bluegrass infestation than sulfentrazone alone. In another study, the combination of overseeding and a fungal control agent reduced dandelion and white clover (*Trifolium repens* L.) populations more than overseeding or the fungal control agent applied alone (Abu-Dieyeh and Watson 2007). These studies suggest the combination of overseeding and herbicides may provide greater dallisgrass control than overseeding or herbicides applied alone; however, this has not been evaluated.

HPPD-INHIBITING HERBICIDES

The discovery of 4-hydroxyphenyl-pyruvate dioxygenase (HPPD) inhibiting herbicides began in 1977 when scientists at the Western Research Center in California observed the allelopathic effects of the bottlebrush plant (*Callistemon citrinus* (Curtis) Skeels) (Beaudegnies et al. 2009). This allelopathic compound was extracted and identified to be leptospermone (Beaudegnies et al. 2009). After years, and many modifications to leptospermone, Zeneca (Syngenta) released the first HPPD-inhibiting herbicide, sulcotrione, in 1993 (Beaudegnies et al. 2009).

Currently, mesotrione, topramezone, and tembotrione are all herbicides that inhibit activity of HPPD and are registered for use in maize (*Zea mays* L.) (Anonymous 2009a; Anonymous 2007; United States EPA 2007; Grossmann and Ehrhardt 2007;

Mitchell et al. 2001; Santel 2009; Senseman 2007). Blockage of this enzyme prevents formation of homogentisate, an essential precursor for the production of plastoquinones and tocopherols (Lindblad et al. 1970; Whistance and Threlfall 1970). Plastoquinone (PQ) is required to shuttle electrons through the Q_B and Q_A sites of photosystem II and in the function of a light harvesting chlorophyll a/b binding protein responsible for distributing energy between photosystem II (PSII) and I (PSI) (Allen et al. 1981; Ort 1986). PQ is also essential to the formation of phytoene desaturase (PDS) as demonstrated by Norris et al. (1995) using PDS deficient *Arabidopsis* mutants. PDS is required to de-saturate the carotenoid precursor phytoene (Mayonado et al. 1989). The C_{40} phytoene is synthesized from condensation of two molecules of the C_{20} isoprenoid geranylgeranyl diphosphate (Figure 1.2). Phytoene is catalyzed by PDS to form ξ-carotene, which is further desaturated to form lycopene, and then cyclized to form α-carotene or β-carotene (Buchannon et al. 2000). Formation of lutein and lutein-5,6-epoxide (epoxylutein) from β-carotene are important in protecting PSII (Pogson et al. 1996). Xanthophyll cycle pigments, violaxanthin (V), zeaxanthin (Z) and antheraxanthin (A), formed from ξ-carotene are involved in light-harvesting and non-photochemical quenching (NPQ). Under excess light (photon-flux density (PFD)) and subsequent reduction of intrathylakoid pH, V is reversibly converted to Z and A in a de-epoxidase reaction catalyzed by violaxanthin de-epoxidase. As PFD decreases and intrathylakoid pH becomes neutral, Z and A are converted back to V through an epoxidase reaction (Demming-Adams et al. 1996). This response has been reported in creeping bentgrass (McElroy et al. 2006). The de-epoxidation of V to Z and A is essential to protect photosystems from excess light (Demming-Adams 1990). Substantiating the connection

between xanthophyll cycle pigments and photoprotection, plants subjected to high irradiance accumulate more total xanthophyll cycle pigments than plants grown under low irradiance (McElroy et al. 2006; Thayer and Björkman 1990). Furthermore, a tomato mutant over-expressing a violaxanthin-de-epoxidase gene causing elevated levels of Z and A displayed a slower decrease in PSII quantum yield under chilling and light stress and an increase in NPQ when compared to the wild-type (Han et al. 2010). This mutant also displayed quicker PSII quantum yield recovery (F_v/F_m) after light and chilling stress ceased (Han et al. 2010).

There is, perhaps no better demonstration of the importance of carotenoids and xanthophyll cycle pigments in the role of photoprotection than the lethality that results from application of a HPPD inhibiting herbicide to a susceptible plant. Sulcotrione decreased total chlorophyll and carotenoids in cucumber (*Cucumis sativus* L.) and soybean (*Glycine max* (L.) Merr.) (Kim et al. 2002; Mayonado et al. 1989). Reduction of total chlorophyll and carotenoids with mesotrione has also been reported on large crabgrass and common bermudagrass (McCurdy et al. 2009b; Brosnan et al. 2010c). This reduction in pigments mirrored an increase in percent visual bleaching also reported by McCurdy et al. (2009b).

Mesotrione was introduced in 2008 as Tenacity® for use in turfgrass on golf courses and sod farms (Anonymous 2008b). Control or suppression of common turfgrass weeds, such as creeping bentgrass, nimblewill (*Muhlenbergia schreberi* J.F. Gmel.) and crabgrass (*Digitaria* spp.) has been reported with mesotrione (Beam, et al. 2006; Giese et al. 2005; Jones and Christians 2007; Willis et al. 2006; Willis et al. 2007). Safety on newly seeded and established stands of tall fescue (*Festuca arundinacea* Schreb.),

perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (Poa pratensis L.), hybrid bluegrass (*Poa pratensis* L. x *Poa arachnifera* Torr.) and centipedegrass (*Eremochloa ophiuroides* Munro. (Kunz)) contributes to mesotrione utility in turfgrass (Beam et al. 2006; Borger et al. 2009; Jones and Christians 2007; McElroy and Walker 2009; McElroy et al. 2007; Willis et al. 2006). While not described in turfgrass, mesotrione selectivity in maize results from more rapid metabolism in tolerant species (Mitchell et al. 2001).

Both foliar and root absorption influence mesotrione efficacy. McCurdy et al. (2009a) reported greater control of yellow nutsedge (*Cyperus esculentus* L.) and large crabgrass (*Digitaria sanguinalis* L. Scop.) with soil applied mesotrione, as compared to foliar-only and soil + foliar applications. However, soil-only treatments were applied by pipetting 10 mL of mesotrione-water solution to each pot, while soil + foliar and foliar-only treatments were applied using a standard CO_2 powered flat-fan nozzle at a carrier volume of 0.27 mL per pot and were not watered until 7 DAT. While the research of McCurdy et al. (2009a) indicates mesotrione is soil active and root absorbed, differences in application method prevent the relative contributions of foliar and root absorption from being determined. Additionally, the influence of relative humidity (RH) and air temperature were not considered by McCurdy et al. (2009a). Johnson and Young (2002) reported that GR_{50} (the herbicide does required to reduce biomass by 50%) values for large crabgrass were seven times higher when mesotrione was applied at 18 C compared to 32 C; additionally, GR_{50} values for large crabgrass were two times higher for applications at 30% RH compared to 85%. Goddard et al. (2010) reported a similar response on smooth crabgrass with greater mesotrione induced injury at 90% RH than

50%. At 90% RH, smooth crabgrass injury from foliar-only applications of mesotrione was not different from that of soil + foliar applications 3, 7 and 14 DAT. Applied at 50% RH, greater smooth crabgrass injury and less dry weight was reported 14 and 21 DAT from soil-only applications as compared to those made only to foliage. These findings illustrate that the contribution of soil and foliar mesotrione uptake is dependent upon environmental conditions. Though information describing the environment within a turfgrass canopy is limited, data taken from a study examining the influence of canopy microenvironments on bacterial populations measured RH levels >90% for greater than 12 hours daily in tall fescue plots in full sun (Giesler et al. 2000). This suggests RH within a turfgrass canopy may be high enough for significant foliar activity of mesotrione.

Factors influencing soil activity of mesotrione include soil organic matter and pH. Mesotrione half-life was reported by Dyson et al. (2002) to range 4.5 to 32 days with soil/water adsorption coefficient (Kd) values ranging from 0.13 to 5.0. Both half-life and Kd values were primarily influenced by soil pH and to a lesser extent, organic matter. Half-life and Kd values demonstrated negative and positive relationships with soil pH and soil organic matter, respectively (Dyson et al. 2002).

Topramezone is a HPPD-inhibiting herbicide belonging to the pyrazolone chemical class (Grossman and Ehrhardt 2007). In field studies, topramezone provides > 95% control of redroot pigweed (*Amaranthus retroflexus* L.), common ragweed (*Ambrosia artemisiifolia* L.) and common lambsquarter (*Chenopodium album* L.) at 50 g ai/ha, barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv) and yellow foxtail (*Setaria glauca* (Poir.) Roem & Schult) at 67 g ai/ha in maize, and greater than 90% control of

star-of-bethlehem (*Ornithogalum umbellatum* L.) at 37 g ai/ha when combined with the PSII inhibiting herbicide bromoxynil (Brosnan et al. 2010b; Gorsic et al. 2008). Relative to mesotrione, topramezone exhibits greater efficacy on annual grassy weeds and is less injurious to corn hybrids (Bollman et al. 2008). In turfgrass, topramezone has more activity on common bermudagrass than mesotrione (Brosnan et al. 2010c). When treated with topramezone (0.018, 0.025, and 0.038 kg/ha) common bermudagrass displayed greater reductions in total chlorophyll and carotenoid pigments 7, 14, and 21 DAT compared to treatment with mesotrione (0.28, 0.35, and 0.42 kg/ha) (Brosnan at al. 2010c). Similar to mesotrione, Kentucky bluegrass and tall fescue tolerance to topramezone has also been observed (Willis and Askew 2008).

Enzyme assays using the 4-HPPD protein from maize and giant foxtail (*Setaria faberi* Herrm.) show that topramezone is 10 times more effective than sulcotrione in 4-HPPD inhibition, establishing topramezone as a potent inhibitor of 4-HPPD (Grossman and Ehrhardt 2007). Enzyme assays have also illustrated that topramezone selectivity is, in part, related to 4-HPPD sensitivity, as giant foxtail (a susceptible species) 4-HPPD is more sensitive than maize (a tolerant species) 4-HPPD (Grossman and Ehrhardt 2007). Research with [^{14}C] topramezone indicated that selectivity is not a result of differential absorption and translocation, rather, more rapid metabolism of topramezone in maize. Adjuvants have also been shown to result in greater than 15 fold improvements in topramezone absorption (Grossman and Ehrhardt 2007). Young et al. (2007) reported methylated seed oil increased activity of topramezone more than crop oil concentrates or nonionic surfactants. Similar to mesotrione, lower air temperatures and reduced relative

humidity negatively affect absorption of [^{14}C] topramezone (Grossman and Ehrhardt 2007).

The triketone herbicide tembotrione is the newest HPPD-inhibiting herbicide (Santel 2009). Tembotrione is formulated with the safener isoxadifen-ethyl at concentrations of 44 g/L and 22 g/L, respectively, as Laudis® for broadleaf and grassy weed control in maize (Anonymous 2008a; Santel 2009). With the exception of fall panicum (*Panicum dichotomiflorum* Michx.), control of annual grassy weeds with tembotrione is similar to that of topramezone but greater than mesotrione (Bollman et al. 2008; Zollinger and Reis 2006; Hahn and Stachowski 2007; Waddington and Young 2007). In turfgrass, tembotrione (0.092, 0.184 and 0.276 kg/ha) and topramezone (0.018, 0.025, and 0.038 kg/ha) are reported to have similar activity against common bermudagrass (Brosnan at al. 2010c).

Bollman et al. (2008) demonstrated most corn hybrids have greater tolerance to tembotrione + isoxadifen-ethyl than topramezone or mesotrione. Willis and Askew (2008) reported tembotrione caused greater injury to perennial ryegrass and less injury to zoysiagrass (*Zoysia japonica* Steud.) than mesotrione and topramezone; otherwise, tolerance of other turfgrass species to tembotrione has been reported to be similar to that of mesotrione and topramezone (Willis and Askew 2008).

While mesotrione activity against dallisgrass has been reported, minimal information regarding topramezone and tembotrione efficacy for dallisgrass control is available. Compared to mesotrione, the greater activity of topramezone and tembotrione against most weed species and similar tolerance of turfgrass suggests they may be more effective in controlling dallisgrass than mesotrione.

CHLOROPHYLL FLUORESCENCE AS A METHOD OF DETECTING HERBICIDE STRESS

Fluorescence, which occurs in about 10^{-8} seconds, is the process by which light is absorbed and re-emitted at a different (usually longer) wavelength (Herman et al. 2010). In chlorophyll fluorescence, most of the energy from absorbed light is transferred for use in photochemistry or to non-photochemical quenching (NPQ) (Maxwell and Johnson 2000). The remaining energy is re-emitted at a longer wavelength than absorbed light, facilitating quantification (Baker 2008). As a result, multiple researchers have supported mesotrione visual bleaching/injury assessments with chlorophyll fluorescence data, particularly assessments of photochemical efficiency (F_v/F_m) (Goddard et al. 2010; McCurdy et al. 2009b; McCurdy et al. 2008; McElroy and Walker 2009). F_v/F_m is determined by subtracting F_o, the minimal level of fluorescence, from F_m, the maximum level of fluorescence and dividing this difference by F_m (Figure 1.3). This ratio is indicative of the quantum yield of PSII photochemistry and when photorespiration is absent, it can be directly proportional to CO_2 assimilation (Genty et al. 1989).

The investigation into practical applications of chlorophyll fluorescence measurement began in 1931 when H. Katusky and A. Hirsch discovered fluorescence of illuminated dark-adapted leaves correlated with CO_2 assimilation (Maxwell and Johnson 2000). Later, Butler developed a model explaining the three fates of PSII excitation energy to be photochemistry, heat loss through NPQ, and fluorescence (Baker 2008). This leads to the logical conclusion that fluorescence could be used to measure changes in photochemistry and heat dissipation if constants for heat loss and fluorescence are

unchanged. Since constants for heat dissipation and photochemistry are variable (Kramer et al. 2004; Krause and Jahns 2004), PSII photochemistry can only be estimated if fluorescence quenching from photochemistry and NPQ are determined. This can be achieved *in-vitro* through addition of PSII inhibitors that stop photochemical quenching, completely reducing Q_A. Use of this technique is not possible in the field due to its time-consuming nature and plant lethality.

This inconvenience led to the discovery that exposing leaves to a short (1s) flash of bright light completely reduces Q_A making it possible to determine the contribution of photochemical and NPQ to fluorescence (Bradbury and Baker 1981; Bradbury and Baker 1984.). Thus, the fluorescence yields before and after a flash of light can be used to determine quantum yield of PSII (Genty et al. 1989) (Figure 1.3). Under field conditions, ambient light can contribute to fluorescence, affecting measurement. To circumvent this problem, plants can be dark-adapted or a modulated fluorometer can be used. Modulating the pulse of light at high frequency allows detection of fluorescence emitted from the fluorometer light source only (Maxwell and Johnson 2000). For this reason, many portable fluorometers use modulated excitation (Maxwell and Johnson 2000).

Widely used in studies involving plant stress, chlorophyll fluorescence measurement has been shown to detect stress from PSII-inhibiting herbicide applications before the appearance of visual symptoms (Hiraki et al. 2003; Percival 2005). Similar findings have been reported with the herbicides glyphosate, imazapyr, asulam and diclofop-methyl causing decreases in F_v/F_m only 6 h after application (Barbagallo et al. 2003). These findings suggest chlorophyll fluorescence measurement may be valuable in detecting stress after application of HPPD-inhibiting herbicides. To this effect, multiple

researchers have used them to supplement visual assessments of HPPD-inhibiting herbicide injury (Goddard et al. 2010; McCurdy et al. 2009; McCurdy et al. 2008; McElroy and Walker 2009). Despite the prevalence of F_v/F_m as an evaluation method in published literature, relationships between F_v/F_m and carotenoid pigment concentrations after application of HPPD-inhibiting herbicides have not been determined.

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OBJECTIVES AND JUSTIFICATION

PROJECT 1: DALLISGRASS CONTROL IN TALL FESCUE

The prevalence of dallisgrass in the southeastern United States, EPA restricted use of MSMA and ineffectiveness of present control methods warrants investigation of innovative control programs involving alternative herbicides, optimum application timings, and combinations of chemical and cultural control methods. Given the seasonal variability in dallisgrass control with fluazifop that has been reported, a more complete investigation to determine optimum application timing is warranted.

Objectives:

- Evaluate the efficacy fluazifop, mesotrione, topramezone and tembotrione for dallisgrass control in tall fescue when applied alone and in mixtures with one another.
- Determine the effect of 4 GDD-based application timings and one cooling degree day* (CDD) based timing on the efficacy of these herbicides for dallisgrass control.
- Determine if fall or spring overseeding of tall fescue affects the efficacy of the aforementioned herbicides for dallisgrass control when applied at GDD-based application timings.

*CDD = T_{base} - [(T_{max}- T_{min})/2], where T_{max} is the daily maximum air temperature, T_{min} is the daily minimum air temperature and T_{base} is the minimum optimal growing temperature.

PROJECT 2: EFFECTS OF HPPD-INHIBITING HERBICIDES ON BERMUDAGRASS

Given that HPPD-inhibiting herbicides indirectly inhibit carotenoid production in susceptible species, herbicidal activity can be determined through quantification of chlorophyll and carotenoid pigments. Direct quantification of carotenoid pigments after treatment with HPPD-inhibiting herbicides is a laborious and time-consuming process (M.T. Elmore, personal observation). Chlorophyll fluorescence measurement and visual assessment of tissue bleaching are methods of rapidly assessing HPPD-inhibiting herbicide activity. Despite their prevalence as evaluation methods in published literature, their relationship with pigments concentrations after application has not been determined.

Objectives:

- Use regression analysis to determine the suitability of chlorophyll fluorescence and visual ratings for detecting differences in leaf tissue pigments after treating 'Riviera' common bermudagrass and 'Tifway' hybrid bermudagrass with mesotrione, topramezone and tembotrione.

PROJECT 3: NITROGEN-ENHANCED EFFICACY OF HPPD-INHIBITING HERBICIDES FOR SMOOTH CRABGRASS CONTROL

Efficacy of the HPPD-inhibiting herbicides mesotrione and topramezone is reduced as smooth crabgrass matures. Increased efficacy of mesotrione and topramezone at greater tiller stages would benefit turfgrass managers. Improvement in herbicide efficacy from nitrogen fertilization has been shown to be species and herbicide specific. There are minimal data describing the effects of nitrogen on the efficacy of HPPD-inhibiting herbicides for smooth crabgrass control.

Objectives:

- Determine if nitrogen enhances efficacy of mesotrione and topramezone for smooth crabgrass control in the field.
- Determine effects of nitrogen on the dose-responses of mesotrione and topramezone applications to smooth crabgrass in a greenhouse.
- Determine effects of nitrogen on smooth crabgrass chlorophyll and carotenoid tissue pigments following treatment with mesotrione and topramezone

Figure 1.1. Dallisgrass (Paspalum dilatatum Poir.) in bermudagrass (Cynodon spp.) turf.

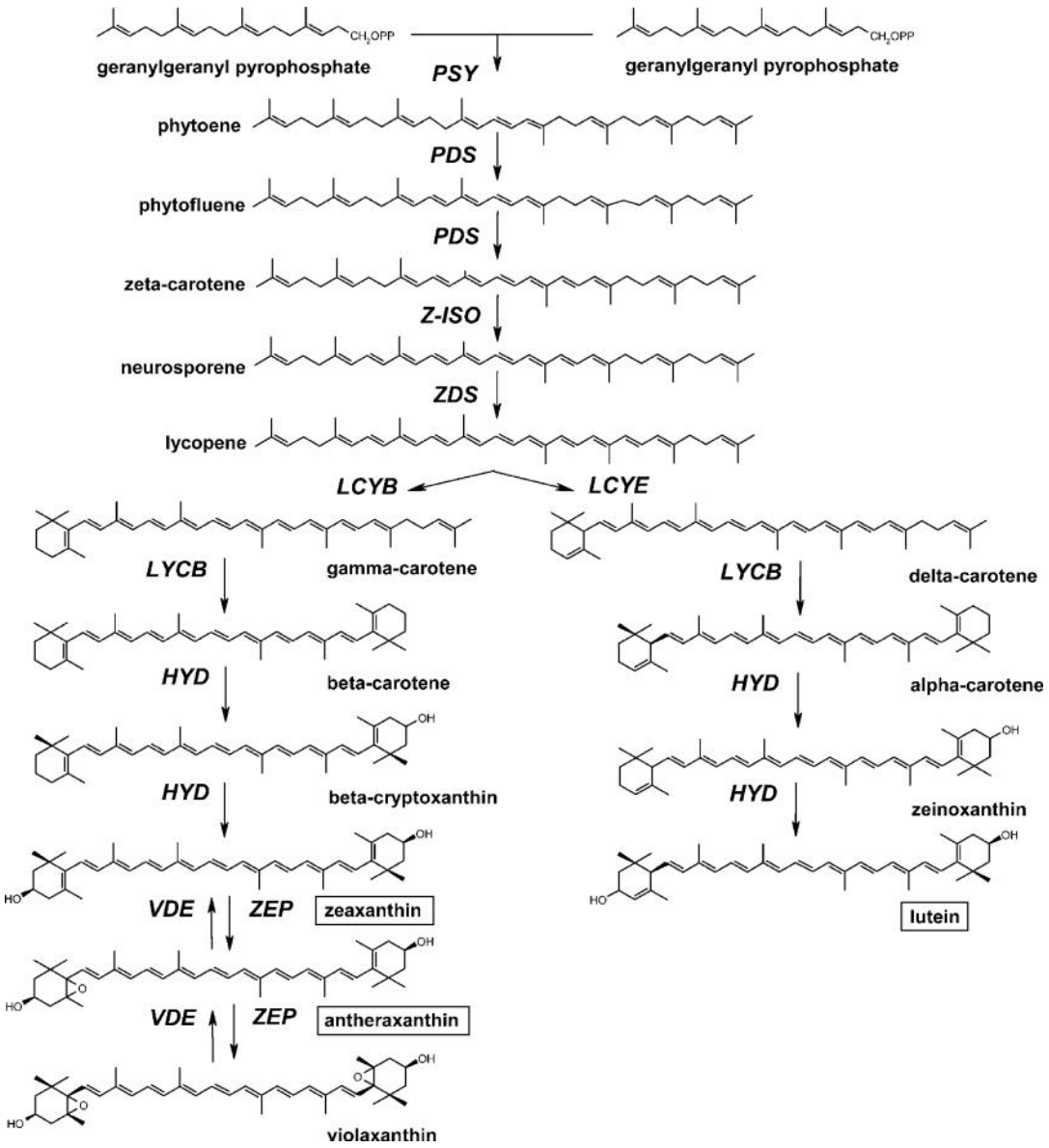

Figure 1.2. Simplified carotenoid biosynthetic pathway. Enzymatic reactions are depicted using solid arrows accompanied by the enzyme abbreviations in capital italics. Enzyme abbreviations: PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ξ-carotene isomerase; ZDS, ζ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; HYD, carotene hydroxylase (both β-ring and ε-ring hydroxylases); ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase. From Kopsell et al. (2009).

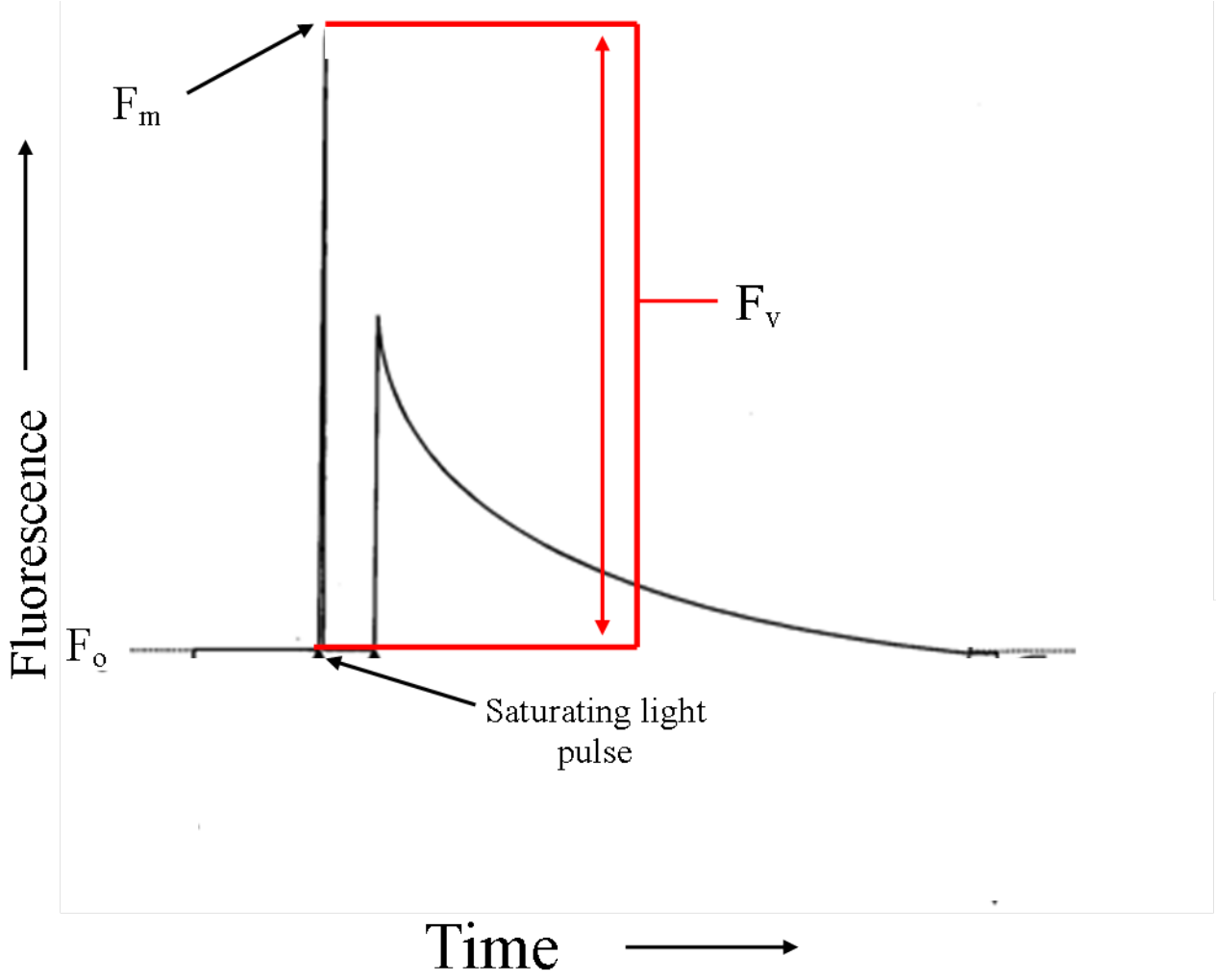

Figure 1.3. Typical chlorophyll fluorescence trace. A measuring light is switched on to determine F_o. This is followed by a brief (1s) saturating pulse of visible light, at which time F_m is measured. The difference between F_o and F_m is termed F_v. Modified from Maxwell and Johnson (2000).

CHAPTER 1

METHODS OF ASSESSING BERMUDAGRASS (*CYNODON DACTYLON* L.) RESPONSES TO HPPD-INHIBITING HERBICIDES

This chapter is based on a paper published by Matthew Elmore, James Brosnan, Dean Kopsell and Gregory Breeden:

Elmore, M.T., J.T. Brosnan, D.A. Kopsell and G.K. Breeden. 2011. Methods of Assessing Bermudagrass (*Cynodon dactylon* L.) Responses to HPPD Inhibiting Herbicides. Crop Science. 51:2840-2845.

My primary contributions to this paper include (i) Conducting the experiments, (ii) processing, analyzing and interpreting data, (iii) reading literature, (iv) writing the manuscript.

ABSTRACT

Mesotrione, topramezone, and tembotrione inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme integral to carotenoid biosynthesis. Research was conducted to evaluate the efficacy of visual bleaching (VB) and chlorophyll fluorescence yield (F_v/F_m) measurements for estimating common bermudagrass [Cynodon dactylon (L.) Pers.] carotenoid and chlorophyll concentrations following mesotrione (0.28, 0.35, and 0.42 kg ha^{-1}), topramezone (0.018, 0.025, and 0.038 kg ha^{-1}), and tembotrione (0.092, 0.184, and 0.276 kg ha^{-1}) applications. Measurements of VB and F_v/F_m were evaluated 3, 7, 14, 21, 28, and 35 days after application (DAA). Leaf tissues were sampled on the same dates and assayed for chlorophyll and carotenoid compounds using HPLC methodology. Carotenoid and total chlorophyll concentrations were regressed upon VB and F_v/F_m on each evaluation date. While significant ($P \leq 0.05$) relationships were

detected on each date, variation explained by linear regression was modest ($R^2 < 0.65$); thus, neither VB nor Fv/Fm assessments are good predictors of carotenoid and chlorophyll concentrations after HPPD inhibiting herbicide treatment. Comparisons of R^2 values for VB and F_v/F_m data suggest no advantage in using F_v/F_m in place of VB measurements when evaluating HPPD inhibiting herbicide activity.

INTRODUCTION

Mesotrione [2-(4-Mesyl-2-nitrobenzoyl)-1,3-cyclohexanedione], topramezone [3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-nethyl-1H-pyrazol-4-yl)methanone], and tembotrione [1,3-cyclohexanedione, 2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl]- (9Cl)] are inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD; EC 1.13.11.27) (Santel, 2009; Senseman, 2007). HPPD inhibition indirectly inhibits production of carotenoid pigments such as lutein, β-carotene, and xanthophyll cycle pigments violaxanthin, zeaxanthin, and antheraxanthin (Sandmann, 2001; Demmig-Adams et al., 1996). Bound to light-harvesting complexes, carotenoids are involved in both light harvesting for photosynthesis and photoprotection (Demmig-Adams et al., 1996; Demmig-Adams and Adams, 1996; Gilmore et al., 1995; Niyogi, 1999; Thayer and Björkman, 1990). Susceptible species treated with HPPD inhibiting herbicides lack sufficient concentrations of carotenoids required to quench singlet- and triplet-state oxygen and dissipate excess photosynthetic active radiation energy as heat, a process termed non-photochemical quenching (NPQ) (Demmig-Adams and Adams, 1996; Niyogi, 1999). Insufficient concentrations of carotenoid pigments results in leaf whitening, subsequent necrosis, and eventual plant mortality in susceptible species (Lee, 1997).

Mesotrione is registered for weed control in turfgrass and maize (*Zea mays* L.), while topramezone and tembotrione are registered for application in maize (Anonymous, 2009; Anonymous, 2008a; Anonymous, 2008b; Anonymous, 2007) and are being researched for use in turfgrass and ornamentals (Armel et al., 2009; Brosnan et al., 2011). Mesotrione can control or suppress common turfgrass weeds, such as creeping bentgrass

(*Agrostis stolonifera* L.), nimblewill (*Muhlenbergia schreberi* J.F. Gmel.), and crabgrass (*Digitaria* spp.) (Beam et al., 2006; Giese et al., 2005; Jones and Christians, 2007; Willis et al., 2006; Willis et al., 2007). Safety on newly seeded and established stands of tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.), hybrid bluegrass (*Poa pratensis* L. x *Poa arachnifera* Torr.), and centipedegrass [*Eremochloa ophiuroides* Munro. (Kunz)] also contributes to mesotrione utility in turfgrass (Beam et al., 2006; Jones and Christians, 2007; McElroy and Walker, 2009; McElroy et al., 2007; Willis et al., 2006). Relative to mesotrione, topramezone and tembotrione exhibit similar safety to cool-season turfgrass species, but greater efficacy against many annual grassy weeds (Bollman et al., 2008; Willis et al., 2008).

Carotenoids, particularly xanthophyll cycle pigments zeaxanthin and antheraxanthin, function in NPQ, which affects chlorophyll fluorescence (Demmig-Adams and Adams, 1996; Müller et al., 2001). Widely used in investigations evaluating the effects of abiotic stress, F_v/F_m measurements can also detect effects induced by mesotrione and other herbicides that directly affect photosynthesis (Hiraki et al., 2003; Kocurek et al., 2009; Maxwell and Johnson, 2000; Percival, 2005). Reductions in F_v/F_m occur as soon as 6 h after herbicide treatment, long before the appearance of visual symptoms (Barbagallo et al., 2003). As a result, multiple researchers have used F_v/F_m assessments to supplement visual ratings of mesotrione activity in field and greenhouse studies (Goddard et al., 2010; McCurdy et al., 2009; McCurdy et al., 2008; McElroy and Walker, 2009). However, direct relationships between F_v/F_m and carotenoid pigment

concentrations following HPPD inhibiting herbicide treatment to turfgrass have not been reported.

Researchers have correlated quantitative evaluations of color (tristimulus colorimetry [L*a*b*] values) with carotenoid pigment concentrations in vegetable crops (Amney and Wilson, 1997; Arias et al., 2000; Berset and Caniaux, 1983; D'Souza et al., 1992; Itle and Kabelka, 2009; Reeves, 1987; Serocznska et al., 2006) with varying levels of success. Simonne et al. (1993) suggested correlations between hue angle and β-carotene concentration in sweet potato (*Ipomoea batatas* Lam.) improve as β-carotene concentration increases. In turfgrass, Yelverton et al. (2009) and Lewis et al. (2010) reported visual assessments of herbicide activity were closely associated with quantitative assessments made using digital image analysis.

Estimating carotenoid and chlorophyll pigment concentrations using F_v/F_m measurements or visual ratings would provide researchers an expeditious alternative to high-pressure liquid chromatography (HPLC) techniques currently used to quantify chlorophyll and carotenoid pigment concentrations following HPPD inhibiting herbicide application (Mayonado et al., 1989; McCurdy et al., 2008; Kopsell et al., 2010). Therefore, the objective of this research was to determine relationships of chlorophyll and various carotenoid pigment concentrations to assessments of F_v/F_m and visual bleaching (VB) following mesotrione, topramezone, and tembotrione applications to 'Riviera' common bermudagrass [*Cynodon dactylon* (L.) Pers.].

MATERIALS AND METHODS

Experiments were conducted during 2009 in a glasshouse at the University of Tennessee (Knoxville, TN). The photoperiod was 13 hours 40 minutes when experiments were initiated and 12 hours 35 minutes at their conclusion; no artificial light was supplied. Daily high air temperatures in the glasshouse ranged from 24 to 33 ºC and averaged 28 ºC. Daily low air temperatures in the glasshouse ranged from 17 to 23 ºC and averaged 20 ºC. Mature 'Riviera' common bermudagrass cores (6 cm diameter, 5 cm depth) were transplanted from the East Tennessee Research and Education Center (Knoxville, TN) to pots (10 cm diameter, 9 cm depth) filled with a peat moss, perlite and vermiculite (55, 25 and 20% v/v, respectively), growing medium[1]. Plants were fertilized with a complete fertilizer[2] (20N:20P_2O_5:20K_2O) following transplant and biweekly thereafter (5.2 kg N ha^{-1}) for the duration of the experiments. Irrigation was applied as needed to prevent moisture stress and encourage optimal growth. Plants were allowed to acclimate for 3 weeks prior to initiation of the experiments. During the acclimation period, plants were clipped twice weekly to a height of 2 cm using hand-shears.

Herbicide treatments consisted of three rates (low, medium, and high) of mesotrione[3] (0.28, 0.35, and 0.42 kg ha^{-1}, respectively), topramezone[4], and tembotrione[5] (0.092, 0.184, and 0.276 kg ha^{-1}, respectively). Low rates represented the maximum-labeled use rate for a single application of each herbicide (Anonymous, 2008a; Anonymous, 2008b; Anonymous, 2007). A non-treated control was also included for comparison. All herbicides were applied with a methylated seed oil surfactant[6] at 0.25% v/v, as MSO reportedly increases the herbicidal activity of mesotrione, topramezone, and tembotrione more than non-ionic surfactants or crop oil concentrates (Young et al.,

2007). Herbicide treatments were applied with 280 L ha^{-1} of water pressurized to 124 kPa using a CO_2-powered boom sprayer containing four flat-fan nozzles[7]. Nozzles were spaced 25 cm apart and maintained 25 cm above the surface while spraying. Immediately prior to herbicide application, plants were clipped for the last time to a 2 cm height.

Percent VB and F_v/F_m were assessed 3, 7, 14, 21, 28, and 35 DAA for all treated and non-treated plants. Visual bleaching was evaluated on a 0 to 100% scale, with 0% representing no tissue bleaching and 100% representing complete bleaching of all leaf tissue. No necrosis was observed at any time. F_v/F_m was measured twice per pot from different newly emerged leaves using a pulse-modulated fluorometer[8]. F_v/F_m was determined by subtracting F_o, the minimal level of fluorescence, from F_m, the maximum level of fluorescence and dividing this difference by F_m (Maxwell and Johnson, 2000).

After VB and F_v/F_m were measured, new leaf tissue growth above 2 cm was harvested with hand-shears, immediately frozen in liquid N, and stored at -80 °C. Leaf tissue pigments were extracted and quantified through HPLC using methods of Kopsell et al. (2007). Carotenoid pigment recovery averaged 72%. For a full description of methods used to extract and quantify leaf tissue pigments, see Brosnan et al. (2011).

Treatments were arranged in a 3 x 3 factorial, randomized complete block, design with three replications. Factors included herbicide (mesotrione, topramezone, and tembotrione) and application rate (low, medium, and high). All data were subjected to a square root transformation prior to analysis (Ahrens et al., 1990). Since interpretations were not different from non-transformed data, the non-transformed means are presented here for clarity.

Effects due to herbicide, application rate, and all possible interactions were determined by subjecting data to analysis of variance using SAS[9]. Two experimental runs were conducted in 2009. No significant treatment-by-experimental run interactions were calculated; thus, combined means are reported. The values for VB, F_v/F_m, and total chlorophyll responses are plotted over DAA with standard error bars presented as a means of statistical comparison. Linear regression analyses were used to determine relationships between chlorophyll and carotenoid pigment concentrations and assessments VB and F_v/F_m using Prism[10] on each observation date (Brosnan et al., 2010; Molulsky and Christopoulos, 2004). Individual observations from all herbicide treatments were pooled and used in regression analyses on each respective date.

RESULTS AND DISCUSSION

Visual Bleaching

Significant herbicide-by-rate interactions were detected in VB data (not presented). This interaction has been described in detail by Brosnan et al. (2011). For the high rates of each herbicide, peak VB measured 22% for mesotrione and 58% for topramezone and tembotrione. Peak VB occurred 7 DAA for all mesotrione-treated plants, regardless of application rate. For tembotrione- and topramezone-treated plants, peak VB occurred 14 DAA for low and medium rates and 21 DAA for the high rate (Figure 2.1).

Total Chlorophyll

The increase in VB coincided with reductions in total chlorophyll for each herbicide. Herbicide-by-rate interactions were detected for total chlorophyll

concentrations (data not presented). Compared to the non-treated control, total chlorophyll concentrations of plants treated with high rates of mesotrione were reduced 44% at 7 DAA. Chlorophyll concentrations for plants treated with high rates of topramezone and tembotrione were reduced 79 and 70%, respectively, compared to the non-treated control 14 DAA (Figure 2.2). For a more detailed description, see Brosnan et al. (2011).

Chlorophyll Fluorescence Yield

Tissue F_v/F_m values for non-treated plants ranged from 0.57 to 0.77 throughout the experiment. Relative to non-treated plants, all herbicide-treated plants displayed a 44 to 74% reduction in F_v/F_m at 3 and 7 DAA (Figure 2.3). Reductions in F_v/F_m were similar to those reported by Kocurek et al. (2009) 3 days after treating *Amaranthus retroflexus* (L.) with mesotrione. Tissue F_v/F_m values for topramezone- and tembotrione-treated plants were lower than those of mesotrione- and non-treated plants 14 DAA. Only F_v/F_m values for topramezone-treated plants were lower than non-treated plants 21 DAA.

Relationships of VB and F_v/F_m with Total Chlorophyll and β-carotene

Significant linear relationships were detected between F_v/F_m values and total chlorophyll and β-carotene concentrations in leaf tissues 7 to 28 DAA (Table 2.1). Similarly, linear relationships were also detected between these pigments and VB 7 to 28 DAA; however, VB was significantly associated with total chlorophyll, lutein, and β-carotene concentrations as soon as 3 DAA. For significant linear relationships of F_v/F_m and VB with total chlorophyll, lutein, and β-carotene R^2 values were modest (≤ 0.62). This response suggests that both F_v/F_m and VB are weak indicators of total chlorophyll, lutein, and β-carotene concentrations following application of mesotrione, topramezone

or tembotrione. Similar responses have been observed using Hunter color values to quantify carotenoids concentrations in vegetable crops low in tissue carotenoids (Simone et al., 1993). Regressions of VB to describe total chlorophyll, lutein, and β-carotene concentrations offered a better fit (higher R^2 values) than those using F_v/F_m measurement, except at 14 DAA.

Relationships of VB and F_v/F_m with Xanthophyll Cycle Pigments

Zeaxanthin and antheraxanthin are xanthophyll cycle pigments that protect antennae chlorophyll by quenching free radicals (Bilger and Björkman, 1990; Demmig-Adams and Adams, 1996; Gilmore et al., 1994). Under excess photon-flux density, intrathylakoid pH is reduced and violaxanthin is reversibly converted to zeaxanthin though the intermediate antheraxanthin via violaxanthin de-epoxidase (Demmig-Adams and Adams, 1996). Linear relationships were detected between F_v/F_m and violaxanthin (7 to 28 DAA) and zeaxanthin (3 to 14, 35 DAA) concentrations (Table 2.2). Using VB data, linear relationships were detected for both violaxanthin (3 to 28 DAA) and zeaxanthin (3 to 14, 35 DAA) concentrations as well. R^2 values for regression equations calculated using F_v/F_m and VB data measured < 0.44 on all dates, illustrating that F_v/F_m and VB data are weak indicators of violaxanthin and zeaxanthin concentrations following applications of mesotrione, topramezone, and tembotrione to common bermudagrass. Reeves (1987) also observed tristimulus color values to be weak indicators ($R^2 = 0.60$) of xanthophyll cycle pigment concentrations in red peppers (*Capsicum annuum L.*). On each date that significant linear relationships were detected using zeaxanthin pigment concentrations, regression equations using VB data offered a better fit (higher R^2 value) than those calculated using F_v/F_m data. Significant relationships detected for violaxanthin

pigment concentrations using VB data offered a better fit than those calculated using F_v/F_m data as well, except at 14 DAA.

Relationships of VB and F_v/F_m with Xanthophyll Cycle Pigment Ratios

The percentage of zeaxanthin + antheraxanthin in the total xanthophyll pigment pool (ZA/ZAV) often increases in leaf tissues under light stress (Demmig-Adams and Adams, 1996). Brosnan et al. (2011) reported this response during the period of peak VB following HPPD inhibiting herbicide treatment. In the current study, significant linear relationships were detected for ZA/ZAV with F_v/F_m and VB on several rating dates; however, R^2 values ranged from 0.15 to 0.34. While this suggests neither F_v/F_m or VB data can be used to estimate ZA/ZAV following HPPD herbicide treatment, regression equations with F_v/F_m data better fit (higher R^2 value) ZA/ZAV data during periods of peak VB (14 to 21 DAA) than those generated using VB (Table 2.2).

Our findings indicate that neither F_v/F_m nor VB evaluation can be used in place of HPLC analyses for estimating bermudagrass chlorophyll and carotenoid pigment concentrations following treatment with the HPPD inhibitors mesotrione, topramezone, and tembotrione. Other researchers demonstrated similar findings in vegetable crops (Amney and Wilson, 1997; Reeves, 1987; Seroczynska et al., 2006). Comparisons of R^2 values for VB and F_v/F_m data suggest no advantage in using F_v/F_m in place of VB measurements to evaluate HPPD inhibiting herbicide activity. Additional research could examine the efficacy of digital image analysis (Karcher and Richardson, 2003) for estimating chlorophyll and carotenoid pigments in turfgrasses treated with HPPD inhibiting herbicides.

SOURCES OF MATERIALS

[1] Super Fine Germinating Mix, Conrad Fafard Inc., Agawam, MA 01001.

[2] Howard Johnson's Triple Twenty Plus Minors, Milwaukee, WI 53204.

[3] Tenacity® 4 SC, Syngenta Professional Products, Greensboro, NC 27409.

[4] Impact® 2.8 SC, Amvac Chemical, Los Angeles, CA 90023.

[5] Laudis® 3.5 SC, Bayer CropScience, Research Triangle Park, NC 27709.

[6] Methylated Seed Oil, Loveland Industries, Greeley, CO 80632.

[7] TeeJet 8002; Spraying Systems Co., Roswell, GA).

[8] OS1-FL, Opti-sciences Inc., Hudson, NH 03051.

[9] Statistical Analysis Software, Inc., Cary, NC 27513.

[10] Prism 5.0 for Mac OSX, GraphPad Software, LaJolla, CA. 92037.

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APPENDIX

TABLES AND FIGURES

Table 2.1. Significance and goodness of fit (R^2) for regression of chlorophyll fluorescence yield (F_v/F_m) and percent visual bleaching (VB) over total chlorophyll, lutein, and β-carotene in leaf tissue of 'Riviera' bermudagrass [*Cynodon dactylon* (L.) Pers.] 3, 7, 14, 21, 28, and 35 days following treatment with mesotrione, topramezone, and tembotrione in 2009.

Assessment Technique	Days After Application (DAA)	Total Chlorophyll		Lutein		β-carotene	
		R^2	P>F	R^2	P>F	R^2	P>F
Chlorophyll Fluorescence Yield (F_v/F_m)	3	NS	NS	NS	NS	NS	NS
	7	0.15	**	0.14	**	0.11	*
	14	0.61	***	0.62	***	0.41	***
	21	0.38	***	0.34	***	0.39	***
	28	0.44	***	0.46	***	0.29	***
	35	NS	NS	0.08	*	NS	NS
Percent Visual Bleaching (VB)	3	0.12	*	NS	NS	0.13	**
	7	0.31	***	0.32	***	0.25	**
	14	0.41	***	0.41	***	0.19	***
	21	0.48	***	0.52	***	0.46	**
	28	0.38	***	0.54	***	0.35	***
	35	NS	NS	0.10	*	NS	NS

*, **, *** Significant at the $P \leq 0.05$, 0.01, and 0.0001 levels, respectively.

NS = non-signfiicant

Table 2.2. Significance and goodness of fit (R^2) for regression of chlorophyll fluorescence yield (F_v/F_m) and percent visual bleaching (VB) over violaxanthin, zeaxanthin and the ratio of zeaxanthin + antheraxanthin to zeaxanthin + antheraxanthin + violaxanthin in leaf tissue of 'Riviera' bermudagrass [*Cynodon dactylon* (L.) Pers.] 3, 7, 14, 21, 28, and 35 days following treatment with mesotrione, topramezone, and tembotrione in 2009.

Assessment Technique	Days After Application (DAA)	Violaxanthin		Zeaxanthin		ZA/ZAV	
		R^2	P>F	R^2	P>F	R^2	P>F
Chlorophyll Fluorescence Yield (F_v/F_m)	3	NS	NS	0.08	*	NS	NS
	7	0.08	*	0.18	**	0.16	**
	14	0.58	***	0.28	***	0.19	**
	21	0.30	***	NS	NS	0.22	**
	28	0.33	***	NS	NS	0.29	***
	35	NS	NS	0.16	**	0.16	**
Percent Visual Bleaching (VB)	3	0.35	***	0.10	*	0.34	***
	7	0.27	***	0.42	***	0.32	***
	14	0.43	***	0.41	***	NS	NS
	21	0.43	***	NS	NS	0.19	**
	28	0.35	***	NS	NS	0.15	**
	35	NS	NS	0.12	*	NS	NS

*, **, *** Significant at the $p \leq 0.05$, 0.01, and 0.0001 levels, respectively.

NS = non-signfiicant

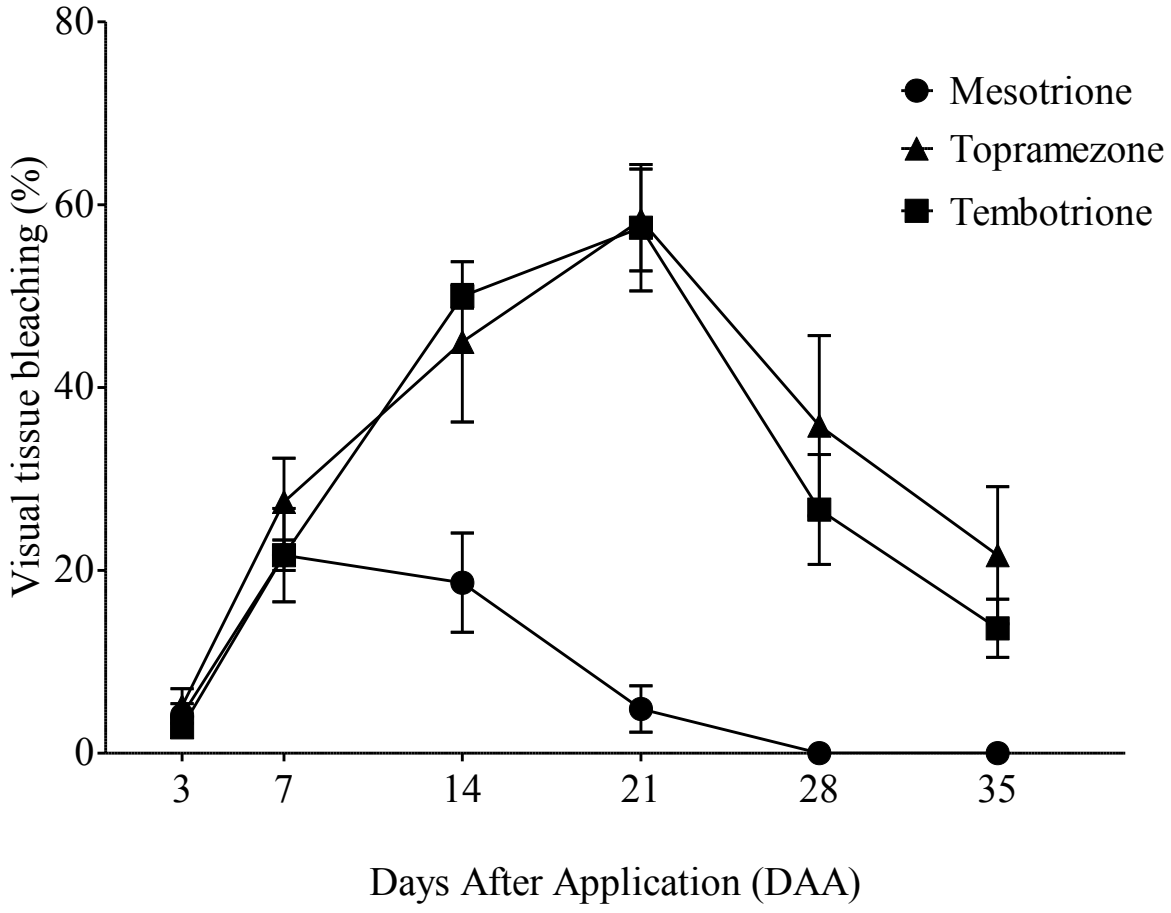

Figure 2.1. Percent visual bleaching (VB) of 'Riviera' bermudagrass [*Cynodon dactylon* (L.) Pers.] leaf tissue 3, 7, 14, 21, 28, and 35 days after mesotrione (0.420 kg ha^{-1}), topramezone (0.038 kg ha^{-1}), and tembotrione (0.276 kg ha^{-1}) application. Error bars indicate standard errors.

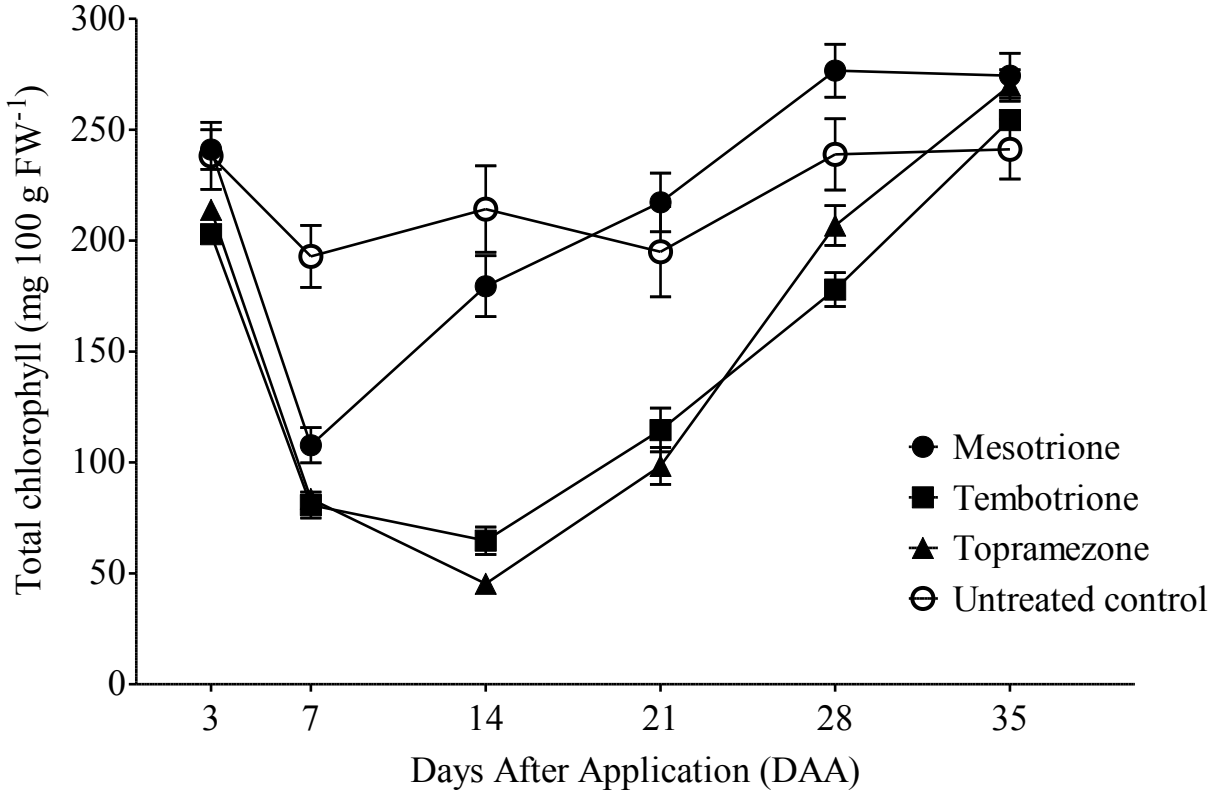

Figure 2.2. Total chlorophyll (chlorophyll *a* and *b*) concentration [mg/100 g fresh weight (FW)] in 'Riviera' bermudagrass [*Cynodon dactylon* (L.) Pers.] leaf tissue 3, 7, 14, 21, 28, and 35 days after mesotrione (0.420 kg ha^{-1}), topramezone (0.038 kg ha^{-1}), and tembotrione (0.276 kg ha^{-1}) application. Error bars indicate standard errors.

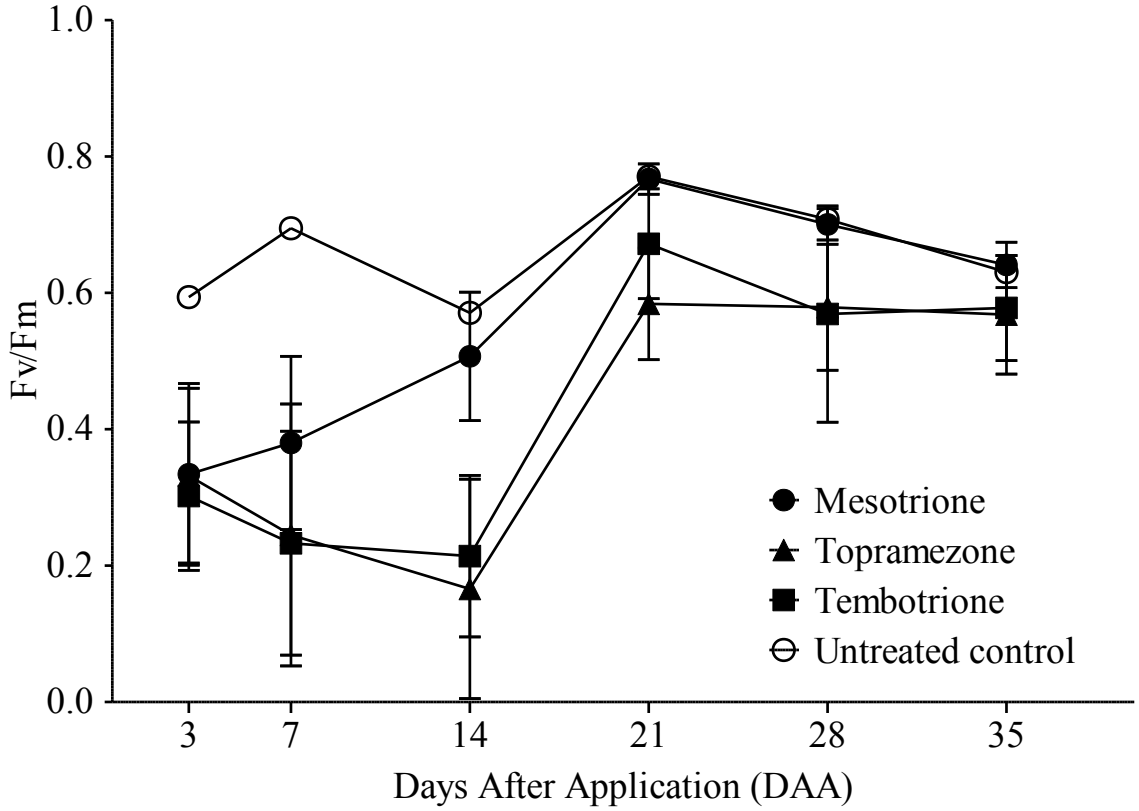

Figure 2.3. Chlorophyll fluorescence yield (Fv/Fm) of 'Riviera' bermudagrass [*Cynodon dactylon* (L.) Pers.] leaf tissue treated with mesotrione, topramezone, and tembotrione at 3, 7, 14, 21, 28, and 35 days after application. Data for each herbicide were combined across application rate. Error bars indicate standard errors.

CHAPTER 2

RESPONSE OF HYBRID BERMUDAGRASS (*CYNODON DACTLYON* X C. *TRANSVAALENSIS*) TO THREE HPPD-INHIBITORS.

This chapter is based on a paper published by Matthew Elmore, James Brosnan, Dean Kopsell, Gregory Breeden and Thomas Mueller:

Elmore, M.T., J.T. Brosnan, D.A. Kopsell, G.K. Breeden and T.C. Mueller. 2011. Response of Hybrid Bermudagrass (*Cynodon dactlyon* x C. *transvaalensis*) to three HPPD-inhibitors. Weed Science. (59:458-463).

My primary contributions to this paper include (i) Conducting the experiments, (ii) processing, analyzing and interpreting data, (iii) reading literature, (iv) writing the manuscript.

ABSTRACT

Mesotrione, topramezone, and tembotrione inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme integral to carotenoid biosynthesis. Research was conducted to evaluate the response of hybrid bermudagrass following mesotrione (280, 350 and 420 g ai ha^{-1}), topramezone (18, 25, and 38 g ai ha^{-1}), and tembotrione (92, 184, and 276 g ai ha^{-1}) applications. Measurements of visual bleaching (VB) and chlorophyll fluorescence yield (F_v/F_m) were evaluated 3, 5, 7, 14, 21, 28, and 35 days after application (DAA). Leaf tissues were sampled on the same dates and assayed for chlorophyll and carotenoid pigments using HPLC methodology. Responses of plants treated with topramezone and tembotrione were similar; these herbicides caused more VB and greater reductions in F_v/F_m, total chlorophyll, lutein, and xanthophyll cycle pigment concentrations than mesotrione 5 to 21 DAA. Increasing mesotrione application rate did not increase VB or

lead to greater reductions in total chlorophyll, lutein or xanthophyll pigment concentrations. Alternatively, increasing topramezone and tembotrione application rates above 18 and 92 g ha^{-1}, respectively, extended VB and pigment reductions. Of the three HPPD-inhibitors tested, topramezone was the most active, as the low (18 g ha^{-1}) rate of topramezone reduced lutein and total xanthophyll pigment concentrations more than the low rate of tembotrione (92 g ha^{-1}) during periods of maximum activity (14 to 21 DAA). No necrosis was observed with any of the treatments, suggesting tank-mixtures of topramezone with other herbicides may be required to provide long-term control of hybrid bermudagrass.

INTRODUCTION

Bermudagrass easily invades cool-season turfgrasses during warmer months due to its aggressive lateral growth from stolons and rhizomes (Rochecouste 1962; Brede 1992). Differences in texture and winter color make bermudagrass [*Cynodon dactylon* (L.) Pers.] infestations in cool-season turfgrass undesirable (Johnson and Carrow 1995). Hybrid bermudagrass has greater density than common bermudagrass, making infestations more problematic (Rochecouste 1962).

Mesotrione, tembotrione, and topramezone are inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD; EC 1.13.11.27) (Grossmann and Ehrhardt 2007; Mitchell et al. 2001; Santel 2009). Inhibition of HPPD indirectly inhibits synthesis of phytoene desaturase, an enzyme critical to carotenoid biosynthesis (Lee et al. 1997). Application of HPPD-inhibiting herbicides to susceptible species results in decreased production of carotenoid pigments such as β-carotene, lutein, violaxanthin and zeaxanthin (Mitchell et al. 2001). Carotenoid pigments are bound to light-harvesting complexes and function as photoprotectants, chemically quenching triplet- and singlet-state oxygen and alternatively dissipating energy as heat in a process termed non-photochemical quenching (NPQ) (Demmig-Adams and Adams 1996; Demmig-Adams et al. 1996; Niyogi 1999). Sub-optimal concentrations of carotenoids result in leaf tissue bleaching (Lee et al. 1997), and possible plant death.

Mesotrione is registered for weed control in turfgrass and maize (*Zea mays* L.), while topramezone and tembotrione are registered for use in maize, but are being examined for use in turfgrass and ornamentals (Anonymous 2009; Anonymous 2008a; Anonymous 2008b; Anonymous 2007; Armel et al. 2009; Brosnan et al. 2011).

Mesotrione, tembotrione and topramezone have efficacy against many broadleaf and grassy weeds and safety to cool-season turfgrasses (Gorsic et al. 2008; Grossman and Ehrhardt 2007; Santel 2009; Willis et al. 2008). With the exception of tembotrione against fall panicum (*Panicum dichotomiflorum* Michx.), efficacy of topramezone and tembotrione against grasses is generally greater than or equal to that of mesotrione (Bollman et al. 2008; Brosnan et al. 2011; Waddington and Young 2007).

Brosnan et al. (2011) reported that topramezone and tembotrione whitened 'Riviera' common bermudagrass leaf tissue to a greater degree than mesotrione. Concomitantly, topramezone and tembotrione caused greater reductions in chlorophyll and carotenoid pigments than mesotrione. All three herbicides increased the percentage of zeaxanthin + antheraxanthin in the total xanthophyll pigment pool 7 days after the peak of visual bleaching. The researchers suggested this increase may be a mechanism of common bermudagrass recovery from HPPD-induced herbicide injury.

Common and hybrid bermudagrass susceptibility to postemergence herbicides varies. Webster et al. (2003) reported variable hybrid and common bermudagrasses response to clethodim and glyphosate. Other researchers have reported differential responses of various common and hybrid bermudagrass cultivars to various pre- and postemergence herbicides (Johnson 1976; Johnson 1985; Johnson 1995; McElroy et al. 2005). The response of hybrid bermudagrass following application of mesotrione, tembotrione and topramezone has not been previously investigated. Therefore, the objective of this research was to evaluate the response of 'Tifway' hybrid bermudagrass to mesotrione, tembotrione and topramezone postemergence applications.

MATERIALS AND METHODS

Plant Culture and Herbicide Treatments. Mature hybrid bermudagrass cores (6 cm diameter by 5 cm depth) were transplanted from the East Tennessee Research and Education Center in Knoxville, TN to pots (10 cm diameter by 6 cm depth) filled with a peat moss (55%), perlite (25%), and vermiculite (20%) growing medium[1] in a glasshouse at the University of Tennessee (Knoxville, TN; 35° 57' N Lat.). The photoperiod was 13 hours 40 minutes when experiments were initiated and 12 hours 35 minutes at their conclusion; no artificial light was supplied. Daily high air temperatures in the glasshouse ranged from 24 to 33 ºC and averaged 28 ºC. Daily low air temperatures in the glasshouse ranged from 17 to 23 ºC and averaged 20 ºC. Plants were maintained at 2 cm using hand-shears and allowed to acclimate for 3 weeks prior to treatment application. Plants were fertilized with a complete fertilizer[2] (20:20:20) following transplanting and biweekly thereafter (5.2 kg N ha^{-1}) for the duration of the experiments.

Herbicide treatments were applied in a factorial treatment combination consisting of three rates (low, medium, and high) of mesotrione[3] (280, 350, and 420 g ha^{-1}, respectively), topramezone[4] (18, 25, and 38 g ha^{-1}, respectively) and tembotrione[5] (92, 184, and 276 g ha^{-1}, respectively). Low rates represented the maximum-labeled use rate for a single application of each herbicide (Anonymous, 2007, 2008a, 2008b). An untreated control was included for comparison. All herbicides were applied with a methylated seed oil surfactant[6] at 0.25% v/v and 280 L ha^{-1} of water. Herbicide treatments were applied using a CO_2-powered boom sprayer containing four flat-fan

nozzles. For a full description of plant culture and treatment application see Elmore et al. (2011).

Data Collection. Percent visual bleaching (VB) and maximum quantum yield of photosystem II (F_v/F_m) were assessed 3, 5, 7, 14, 21, 28, and 35 DAA for all treated and untreated plants. VB was evaluated using a 0 to 100% scale, with 0% representing no tissue bleaching and 100% representing complete bleaching of all leaf tissue. No necrosis was observed at any time. Values of F_v/F_m were measured twice per pot from different newly emerged leaves using a pulse-modulated fluorometer[7], similar to the methods of Goddard et al. (2010). F_v/F_m values were determined by subtracting F_o, the minimal level of fluorescence, from F_m, the maximum level of fluorescence to determine variable fluorescence (F_v), and dividing this difference by F_m (Maxwell and Johnson, 2000). After evaluation at each date, leaf material above 2 cm was harvested, frozen in liquid N_2, and placed on ice for transfer to storage at -80°C. Leaf tissue pigments were extracted and quantified through high-pressure liquid chromatography using previously published methods (Brosnan et al. 2011; Kopsell et al. 2007). Pigments were expressed as mg 100 g fresh weight^{-1} (FW) of hybrid bermudagrass leaf tissue.

Statistical Analysis. Two separate experiments were conducted, each with plants arranged in a randomized complete block design with three replications. Data from untreated plants were excluded to stabilize variance. Data were subjected to ANOVA in SAS[8]. No interactions were detected between experiments; thus, data were combined. When herbicide-by-rate interactions were detected, Fisher's protected least significant difference (LSD) values were used to separate treatment means at the $p \leq 0.05$ level. To illustrate effects of each herbicide over time, means for the high rate of each herbicide are

plotted over harvest interval with standard error values as a means of statistical comparison.

Linear regression analyses were used to determine relationships between chlorophyll and carotenoid pigment concentrations and assessments of VB and F_v/F_m using Prism[9] on each observation date (Elmore et al. 2011; Molulsky and Christopoulos 2004). Data from all herbicide treatments were pooled and used in these regression analyses on each respective observation date similar to Elmore et al. (2011).

RESULTS AND DISCUSSION

Visual Bleaching (VB). Leaf tissue VB was observed from 3 to 14 DAA for the high rate (420 g ha^{-1}) of mesotrione and 3 to 35 DAA for the high rates topramezone (38 g ha^{-1}) and tembotrione (276 g ha^{-1}) (Figure 3.1). Maximum leaf bleaching with mesotrione occurred 7 DAA compared to 14 DAA for topramezone and tembotrione. Except at 7 and 28 DAA, no differences in VB were detected between high rates of topramezone and tembotrione; however, both herbicides resulted in greater VB than the high rate of mesotrione, which supports the findings of Brosnan et al. (2011) on common bermudagrass. High rates of topramezone and tembotrione resulted in as high as 45% and 48% VB, compared to only 11% with mesotrione. These values are lower than those reported by Brosnan et al. (2011) following mesotrione, topramezone, and tembotrione applications to common bermudagrass. Significant herbicide-by-rate interactions in VB were detected 21 to 35 DAA (Table 3.1). VB increased with topramezone rate 35 DAA and tembotrione rate from 21 to 35 DAA; however, no differences in VB were detected with increasing mesotrione rate at these timings.

Chlorophyll Fluorescence Yield (F_v/F_m). Mean F_v/F_m values for untreated plants ranged from 0.52 to 0.73 (Figure 3.2). Compared to the untreated control, the high rate of mesotrione reduced F_v/F_m at 5 to 14 DAA, maximally by 37% compared to the untreated control occurring 5 DAA. These results for hybrid bermudagrass are different than those of Elmore et al. (2011) who reported mesotrione reduced F_v/F_m of common bermudagrass as soon as 3 DAA. Considering that VB was observed on newly emerged leaf tissues, the delay in F_v/F_m reductions could be due to the the slower vertical growth rate of hybrid bermudagrass compared to common bermudagrass (Beard 1973). The high rates of topramezone and tembotrione reduced F_v/F_m similarly and to a greater extent than mesotrione 5 to 28 DAA. Similar to observations of Elmore et al. (2011) on common bermudagrass, the high rates of topramezone and tembotrione maximally reduced F_v/F_m by 72 and 71% compared to the untreated control, respectively, 14 DAA. Herbicide-by-rate interactions in F_v/F_m data were detected 21 DAA (Table 3.1). Increasing mesotrione rate did not affect F_v/F_m. The 276 g ha^{-1} rate of tembotrione reduced F_v/F_m more than the 184 g ha^{-1} rate. The 25 and 38 g ha^{-1} rates of topramezone reduced F_v/F_m more than 18 g ha^{-1}.

Pigment Responses. Increases in VB coincided with decreases in total chlorophyll (chlorophyll *a* + chlorophyll *b*) and carotenoid pigment concentrations (Figure 2.3). This response has been reported by other researchers evaluating the effects of HPPD-inhibitors on common bermudagrass (Brosnan et al. 2011; Elmore et al. 2011; Kopsell et al. 2010). Maximum reductions in total chlorophyll occurred during peak VB regardless of herbicide (Figure 3.3). The high rate of mesotrione reduced total chlorophyll by 26% compared to the untreated control at 7 DAA. By 14 DAA, total chlorophyll

concentrations with the high rate of mesotrione and the untreated control were equal (256 mg 100 g FW^{-1}). Except at 28 DAA no differences were detected between high rates of topramezone and tembotrione, as each reduced total chlorophyll concentrations maximally by 69 and 71% compared to the untreated control, respectively, 14 DAA. Brosnan et al. (2011) observed similar patterns following applications of mesotrione, topramezone and tembotrione to 'Riviera' common bermudagrass. However, greater reductions in total chlorophyll concentrations were observed in this experiment on hybrid bermudagrass.

Herbicide-by-rate interactions detected 14 to 28 DAA for total chlorophyll were similar to those observed in VB data (Table 3.1). No differences were detected between mesotrione rates when evaluated 14 to 28 DAA. Compared to the untreated control, topramezone at 25 and 38 g ha^{-1} reduced total chlorophyll 2.5 times more (74 mg 100 g FW^{-1}) than 18 g ha^{-1} (193 mg 100g FW^{-1}) at 14 DAA. By 21 DAA, only the 38 g ha^{-1} rate of topramezone reduced total chlorophyll more than the 18 or 25 g ha^{-1} rates. While no significant differences were detected between tembotrione rates at 14 DAA, 276 g ha^{-1} of tembotrione reduced total chlorophyll greater than tembotrione at 184 and 92 g ha^{-1} at 21 and 28 DAA, respectively.

Responses of leaf tissue lutein concentrations were similar to those observed with total chlorophyll. The most concentrated carotenoid pigment, lutein, comprised 42 to 49% of total carotenoids in treated plants from 3 to 35 DAA. Treatment with the high rate of mesotrione reduced lutein concentrations by 26% compared to the untreated control 7 DAA (Figure 2.3). High rates of topramezone and tembotrione caused greater reductions in lutein than the high rate of mesotrione 7 to 28 DAA. At 14 DAA, high rates of

topramezone and tembotrione maximally reduced lutein concentrations by 65 and 70% compared to the untreated control, respectively. Herbicide-by-rate interactions were detected in lutein data 14 to 28 DAA (Table 3.2). Few differences in lutein concentrations were detected between rates of mesotrione or topramezone at these timings. Compared to the untreated control, tembotrione rates of 184 and 276 g ha^{-1} decreased lutein at 14 to 28 DAA more than twice that of the 92 g ha^{-1} rate. The low rate of topramezone (18 g ha^{-1}) reduced lutein by 65% (5.3 mg 100 g FW^{-1}) compared to the untreated control at 21 DAA but the medium rate (184 g ha^{-1}) rate of tembotrione was required to reduce lutein by 69% compared to the untreated control on the same date.

Among carotenoid pigments, zeaxanthin is the primary carotenoid responsible for NPQ (Baroli et al. 2003). Violaxanthin is rapidly converted to zeaxanthin via antheraxanthin in response to excess photosynthetic radiation, making xanthophyll cycle pigment concentrations indicators of photoprotective ability (Demmig-Adams et al., 1996). Concentrations of xanthophyll cycle pigments in plants treated with the high rate of mesotrione were reduced by 14% compared to the untreated control 5 DAA, but were not significantly different from the untreated control on any other date (Figure 3.3). Xanthophyll cycle pigment concentrations were similar for topramezone and tembotrione on all dates. High rates of topramezone and tembotrione reduced total xanthophyll pigment concentrations more than mesotrione, by 62 and 70% compared to the untreated-control 14 DAA. Herbicide-by-rate interactions were detected in total xanthophyll data 14 to 28 DAA (Table 3.2). Treatment responses were similar to those observed in lutein data. While both topramezone and tembotrione reduced total xanthophyll pigment

concentrations more than mesotrione, the low rate of topramezone (18 g ha^{-1}) resulted in greater reductions than the low rate of tembotrione (92 g ha^{-1})

The percentage of zeaxanthin and antheraxanthin in the total xanthophyll pigment pool (ZA/ZAV) is often an indicator of leaf tissue acclimation to high photon-flux densities (Demmig-Adams et al. 1995). Brosnan et al. (2011) suggested increases in ZA/ZAV may be an indicator of common bermudagrass recovery from HPPD-inhibiting herbicide injury. In this experiment, large increases in ZA/ZAV during post-application recovery were not observed on hybrid bermudagrass (data not presented).

Regression Analyses. Significant linear regression relationships between VB, F_v/F_m and total chlorophyll, lutein, and violaxanthin were detected for data collected 7 to 28 DAA (data not presented). Similar to Elmore et al. (2011), linear models best-fit (e.g., highest R^2 values) data collected 14 to 21 DAA. R^2 values never exceeded 0.71 on any date, suggesting that neither F_v/F_m nor visual evaluations of tissue bleaching can accurately predict total chlorophyll, lutein, or violaxanthin concentrations after applying HPPD-inhibiting herbicides. Except for violaxanthin data collected 7 DAA, models using VB data offered a better fit (e.g., higher R^2 value) than those using F_v/F_m, suggesting F_v/F_m measurements should not be substituted for visual evaluations.

Plant responses indicate that increasing mesotrione rate from 280 to 420 g ha^{-1} does not increase activity on hybrid bermudagrass. This supports what has been reported on common bermudagrass (Brosnan et al. 2011). Alternatively, increasing tembotrione and topramezone application rate enhanced activity of these herbicides on hybrid bermudagrass.

Topramezone and tembotrione were more active than mesotrione against bermudagrass, reducing leaf tissue pigment concentrations by over 60% compared to the untreated control. No leaf necrosis was observed on any rating date with either herbicide, suggesting neither are able to provide long-term hybrid bermudagrass control with single applications. Occurrence of peak VB and maximum pigment reductions following topramezone and tembotrione application suggest that sequential applications should occur on 14 to 21 day intervals. These findings are similar to those of Brosnan et al. (2011) who suggested topramezone and tembotrione be applied on 14-day intervals to control common bermudagrass. Of the three HPPD-inhibitors tested, topramezone was the most active; the 18 g ha^{-1} rate of topramezone reduced carotenoid tissue pigment concentrations more than the low rate of tembotrione (92 g ha^{-1}) during periods of maximum activity (14 to 21 DAA). Future research should focus on tank-mixing topramezone at 18 g ha^{-1} with other herbicidal modes of action to improve bermudagrass suppression.

SOURCES OF MATERIALS

[1] Super Fine Germinating Mix, Conrad Fafard Inc., Agawam, MA 01001.

[2] Howard Johnson's Triple Twenty Plus Minors, Milwaukee, WI 53204.

[3] Tenacity® 4 SC, Syngenta Professional Products, Greensboro, NC 27409.

[4] Impact® 2.8 SC, Amvac Chemical, Los Angeles, CA 90023.

[5] Laudis® 3.5 SC, Bayer CropScience, Research Triangle Park, NC 27709.

[6] Methylated Seed Oil, Loveland Industries, Greeley, CO 80632.

[7] OS1-FL, Opti-sciences Inc., Hudson, NH 03051.

[8] Statistical Analysis Software, Inc., Cary, NC 27513.

[9] Prism 5.0 for Mac OSX, GraphPad Software, LaJolla, CA. 92037.

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APPENDIX

TABLES AND FIGURES

Table 3.1. Herbicide-by-rate interactions for visual bleaching (VB), chlorophyll fluorescence yield (F_v/F_m)**,** and total chlorophyll concentrations in leaf tissue of 'Tifway' hybrid bermudagrass following mesotrione, tembotrione and topramezone applications.

		Visual bleaching			Fluorescence	Total chlorophyll		
Herbicide[b]	Rate	21 DAA[c]	28 DAA	35 DAA	21 DAA	14 DAA	21 DAA	28 DAA
	g ai ha^{-1}	%			F_v/F_m[a]	mg 100 g FW^{-1}[d]		
Mesotrione	280	0	0	0	0.77	231	302	367
	350	1	0	0	0.79	280	340	353
	420	0	0	0	0.76	257	330	341
Tembotione	18	38	6	4	0.68	193	277	354
	25	40	4	3	0.42	74	194	205
	38	35	6	14	0.52	74	96	191
Topramezone	92	16	1	1	0.56	83	176	333
	184	34	10	2	0.65	120	215	309
	276	44	14	22	0.5	81	121	263
Untreated control		--	--	--	0.77	258	283	345
$LSD_{0.05}$[c]		8	6	8	0.10	69	87	66

[a] F_v/F_m = chlorophyll fluorescence yield
[b] All herbicide treatments applied with a methylated seed oil surfactant at a 0.25% v/v ratio
[c] Abbreviations: days after application, DAA; Fisher's least significant difference, LSD.
[d] Chlorophyll concentration expressed as mg 100 g fresh weight^{-1} (FW) of hybrid bermudagrass leaf tissue

Table 3.2. Herbicide-by-rate interactions for lutein and total xanthophyll cycle (violaxanthin, neoxanthin, and antheraxanthin) pigment concentrations in leaf tissue of 'Tifway' hybrid bermudagrass following mesotrione, tembotrione and topramezone applications.

		Lutein			Total xanthophyll		
Herbicide[a]	Rate	14 DAA[b]	21 DAA	28 DAA	14 DAA	21 DAA	28 DAA
	—g ai ha^{-1}—	mg 100 g FW^{-1}[c]					
Mesotrione	280	13.1	16.5	18.1	8.2	9.8	12.7
	350	15.6	17.9	20.4	10.2	11.7	12.2
	420	14.1	18.1	20.0	9.7	12.0	12.2
Topramezone	18	5.3	8.4	17.5	3.3	5.9	11.4
	25	7.1	11.4	16.3	4.6	7.8	12.4
	38	5.2	6.4	12.9	3.2	4.4	8.9
Tembotrione	92	10.8	14.6	20.6	7.9	9.8	12.9
	184	4.7	9.6	10.4	3.0	6.9	7.1
	276	4.6	5.2	9.0	2.9	3.3	6.1
Untreated control		15.1	16.3	20.3	9.5	9.5	11.8
$LSD_{0.05}$[b]		3.2	4.6	5.4	2.7	3.4	2.5

[a] All herbicide treatments applied with a methylated seed oil surfactant at a 0.25% v/v ratio
[b] Abbreviations: days after application, DAA; Fisher's least significant difference, LSD;
[c] Pigment concentrations expressed as mg 100 g fresh weight^{-1} (FW) of hybrid bermudagrass leaf tissue

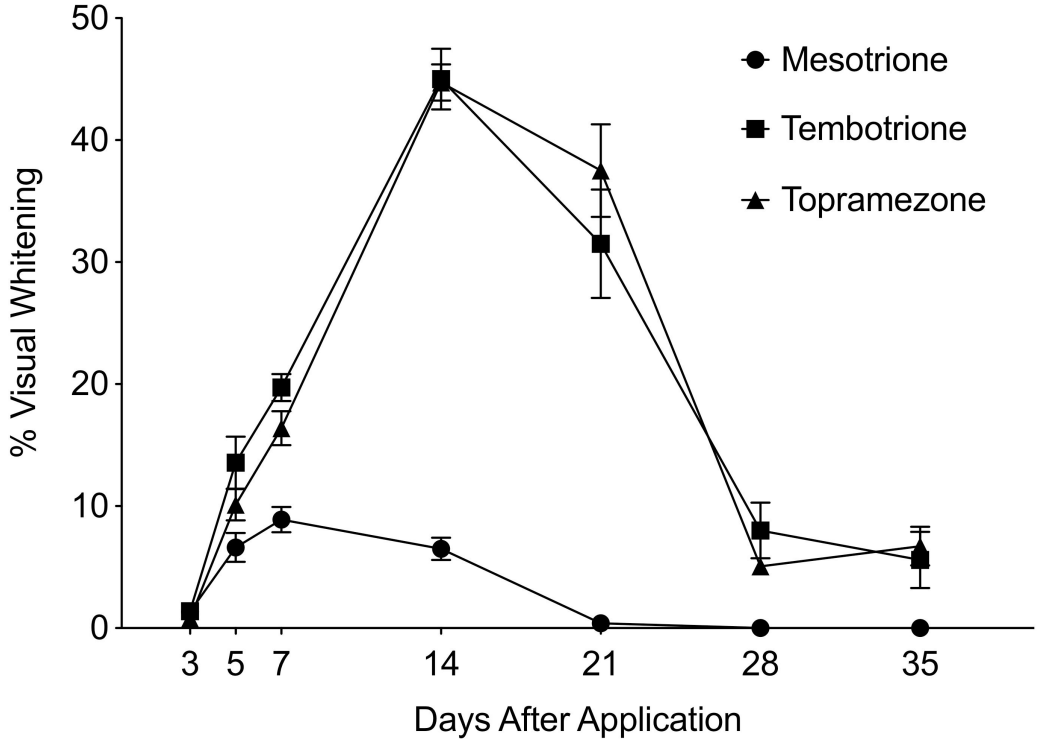

Figure 3.1. Visual bleaching (VB) of 'Tifway' hybrid bermudagrass leaf tissue treated with mesotrione (420 g ha^{-1}), topramezone (38 g ha^{-1}), and tembotrione (276 g ha^{-1}) at 3, 5, 7, 14, 21, 28, and 35 days after application. Error bars indicate standard errors.

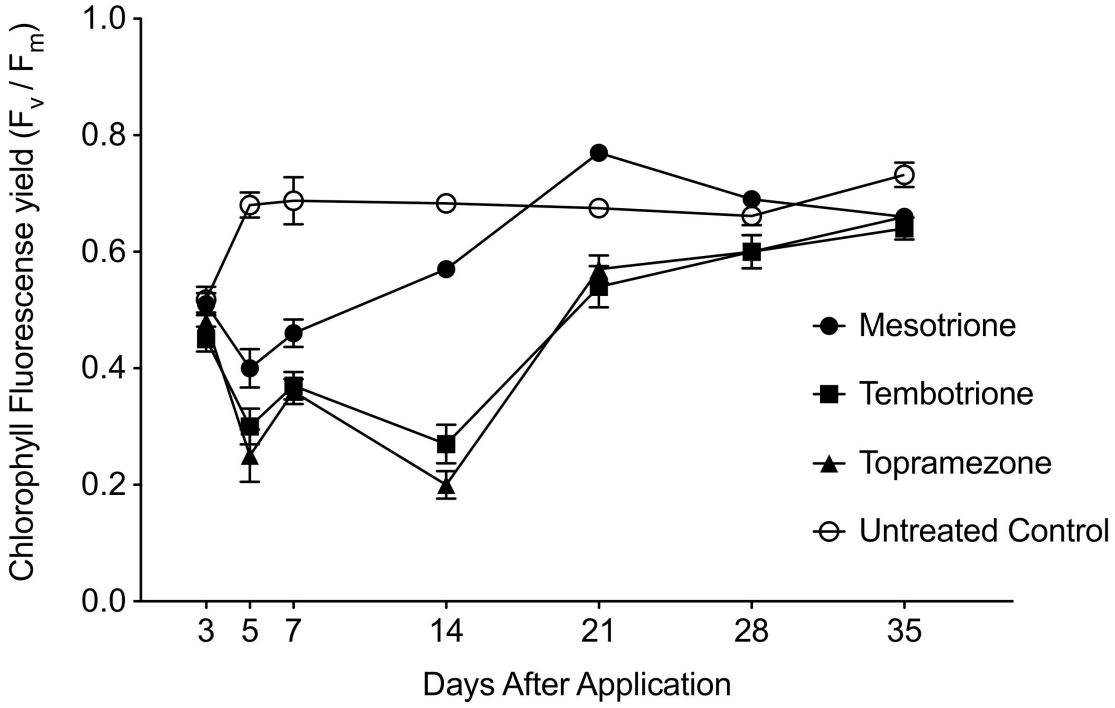

Figure 3.2. Chlorophyll fluorescence yield (F_v/F_m) of 'Tifway' hybrid bermudagrass leaf tissue treated with mesotrione (420 g ha^{-1}), topramezone (38 g ha^{-1}), and tembotrione (276 g ha^{-1}) at 3, 5, 7, 14, 21, 28, and 35 days after application. Error bars indicate standard errors.

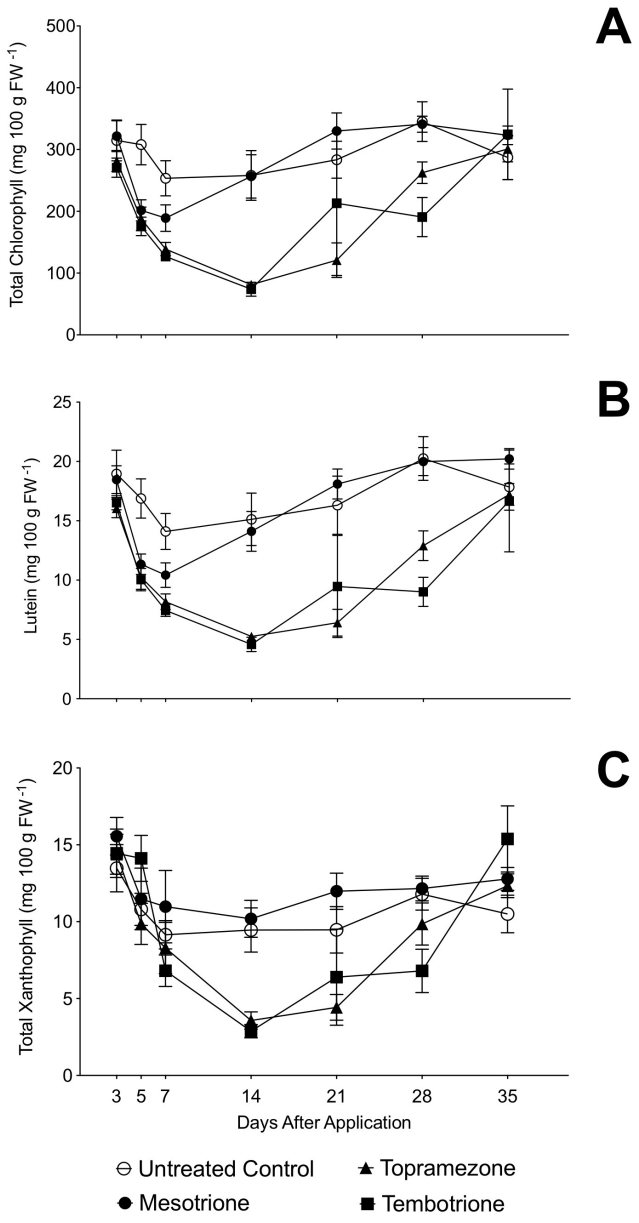

Figure 3.3. Concentrations of (A) total chlorophyll (chlorophyll *a* + chlorophyll *b*), (B) lutein, and (C) xanthophyll cycle (zeaxanthin + antheraxanthin + violaxanthin) pigments (mg 100 g FW^{-1}) in 'Tifway' hybrid bermudagrass leaf tissue treated with mesotrione (420 g ha^{-1}), topramezone (38 g ha^{-1}), and tembotrione (276 g ha^{-1}) at 3, 5, 7, 14, 21, 28, and 35 days after application. Error bars indicate standard errors.

CHAPTER 3

NITROGEN-ENHANCED EFFICACY OF MESOTRIONE AND TOPRAMEZONE FOR SMOOTH CRABGRASS (*DIGITARIA ISCHAEMUM*) CONTROL

This chapter is based on a paper submitted for publication by Matthew Elmore, James Brosnan, Dean Kopsell and Gregory Breeden:

Elmore, M.T., J.T. Brosnan, D.A. Kopsell and G.K. Breeden. 2011. Nitrogen-Enhanced Efficacy of Mesotrione and Topramezone for Smooth Crabgrass (*Digitaria ischaemum*) Control. Weed Science (Submitted: October 12, 2011).

My primary contributions to this paper include (i) Discovering the concept (ii) Design and conducting the experiments, (iii) processing, analyzing and interpreting data, (iv) reading literature, (v) writing the manuscript.

ABSTRACT

The herbicides mesotrione and topramezone inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD) and have efficacy against smooth crabgrass (*Digitaria ischaemum*). Research was conducted to determine the impacts of soil-applied nitrogen (N) fertilizer on the effectiveness of mesotrione and topramezone for smooth crabgrass control. Field experiments in 2010 and 2011 evaluated the efficacy of mesotrione (280 g ha^{-1}) and topramezone (9 g ha^{-1}) for control of multi-tiller smooth crabgrass subjected to five N fertility treatments (0, 12, 25, 37 or 49 kg N ha^{-1}). Greenhouse experiments evaluated the response of smooth crabgrass to mesotrione (0, 70, 140, 280, 560 and 1120 g ha^{-1}) and topramezone (0, 4.5, 9, 18, 36 and 72 g ha^{-1}) with 0 or 49 kg N ha^{-1}. Further research evaluated changes in smooth crabgrass leaf tissue following treatment with mesotrione (280 g ha^{-1}) and topramezone (18 g ha^{-1}) with 0 or 49 kg ha^{-1}. In field experiments, N

increased smooth crabgrass control with mesotrione and topramezone for 8 weeks; however, increasing N rate above 25 kg ha^{-1} did not improve control on any rating date. In dose-response experiments, N application reduced I_{50} values for mesotrione and topramezone by 50 and 65%, respectively, 21 days after treatment (DAT). Reductions in aboveground biomass with both herbicides were greater when applied following N treatment as well. In leaf-response experiments, N decreased new leaf chlorophyll and carotenoid concentrations and new leaf production after treatment with topramezone. Future research should investigate whether increased translocation of these herbicides to meristimatic regions contributed to N-enhanced efficacy.

INTRODUCTION

Smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.) is a problematic weed throughout the United States (Kim et al. 2002). Smooth crabgrass can be selectively controlled by both PRE and POST herbicides (Troll 1962; Jagschitz 1970). Mesotrione and topramezone are inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) with efficacy against smooth crabgrass in cool-season turfgrass (Beam et al. 2006; Johnson et al. 2008; Jones and Christians 2007; Schönhammer et al. 2006; Willis 2008a; 2008b). Efficacy of mesotrione for smooth crabgrass control varies with growth stage. Giese et al. (2005) reported mesotrione applied at 280 g ha^{-1} controlled smooth crabgrass 78% at an early POST timing compared to 26% at a late POST timing. Similarly, Dernoeden and Fu (2008) reported greater smooth crabgrass control at the 4-leaf to 2-tiller stage than the 3- to 8-tiller stage from single applications of mesotrione at 280 g ha^{-1}. Increasing the ability of mesotrione and topramezone to control smooth crabgrass at later tiller stages would be beneficial to turfgrass managers.

Nitrogen (N) fertilization influences the response of weeds to various herbicides. Cathcart et al. (2004) found GR_{50} (the herbicide dose required to reduce biomass by 50%) values increased 3.5-fold for mesotrione against redroot pigweed (*Amaranthus retroflexus* L.) grown under low N compared to high N; however, N status did not influence velvetleaf (*Abulilion theophrasti* Medic.) control with mesotrione. This indicates that plant-N interactions with herbicides are likely species dependent (Cathcart et al. 2004). Brosnan et al. (2010) demonstrated increased control of annual bluegrass (*Poa annua* L.) with flazasulfuron by applying granular N immediately prior to herbicide application. They attributed an increase in control, at least in part, to an increase in

flazasulfuron translocation. Dickson et al. (1990) found activity of fluazifop and glyphosate to increase on oats (*Avena sativa* L. 'Amuri') when they were watered with high N, as opposed low N solution, for 10 days prior to herbicide application. When plants were watered with low N solution 10 days prior to herbicide application and then flushed with high N solution immediately after herbicide treatment, fluazifop and glyphosate activity was not improved.

Providing a physiological basis for N-enhanced glyphosate efficacy, Mithila et al. (2008) reported velvetleaf and common lambsquarters (*Chenopodium album* L.) grown under high soil N displayed increased ^{14}C-glyphosate translocation. Mesotrione and topramezone are phloem-translocated to apical meristems and preferentially bleach developing leaves (Goddard et. al. 2010; Grossman and Ehrhardt 2007; Senseman, 2007; Weinberg et al. 2003). In N deficient plants, N application increases photoassimilate production, new leaf formation, and leaf area index (Wilson and Brown 1983). Application of N may increase herbicide translocation to shoot meristimatic regions, and increase smooth crabgrass bleached leaf formation, thereby increasing control.

Increased mesotrione and topramezone efficacy using urea ammonium nitrate (UAN) or other pH-reducing acid adjuvants has been reported (Grossman and Ehrhardt 2007; LiJuan et al. 2011; Wichert and Pastushok 2000; Idziak and Woznica 2008). However, this is likely a result of increased herbicide absorption, especially in a cation-containing spray solution with neutral to basic pH, as mesotrione and topramezone are weak acids (pK_a 3.12 and 4.06, respectively) (Senseman 2007). There are no reports of enhanced-efficacy of mesotrione and topramezone when N fertilizer is applied to the soil in quantities that significantly affect plant N status.

We hypothesize soil-applied N will enhance efficacy of mesotrione and topramezone for multi-tiller smooth crabgrass control due to greater production of bleached leaf tissue. Research was conducted in 2010 and 2011 at the University of Tennessee to determine the effects of N on the efficacy of mesotrione and topramezone for smooth crabgrass control.

MATERIALS AND METHODS

Field experiments. Research was conducted in 2010 and 2011 on a site naturally infested with smooth crabgrass at the East Tennessee Research and Education Center (Knoxville, TN; 35° 57' N Lat.). Plots were established on a Sequatchie loam soil [Fine-loamy, siliceous, semiactive, thermic humic Hapludult] measuring 6.2 in soil pH and 2.1% in organic matter content. Field trials were conducted in an area of full sunlight, mowed twice weekly at ~5 cm, and irrigated as needed to prevent wilt. At study initiation each year, smooth crabgrass plants had 3- to 5- tillers and covered approximately 50% of untreated plots.

Two herbicides and five N rates were evaluated. Herbicide treatments consisted of mesotrione[1] and topramezone[2] at 280 and 9 g ai ha^{-1}, respectively. Each herbicide included a non-ionic surfactant[3] at 0.25 % v/v and was applied using a CO_2-powered sprayer containing four flat-fan nozzles[4] calibrated to deliver 280 L ha^{-1}. Five rates of N (0, 12, 25, 37 or 49 kg N ha^{-1}) were applied as granular ammonium sulfate (21N-0P_2O_5-0K_2O) and watered in immediately prior to herbicide treatment. An untreated control that received no herbicide or fertilizer treatment was also included for comparison.

Smooth crabgrass control was visually evaluated 4 days after treatment (DAT) and then weekly from 1 to 8 weeks after treatment (WAT) on a 0 (no control) to 100 % (complete kill) scale. To assess turfgrass phytotoxicity, these treatments were applied to a weed-free stand of mature 'Coyote II' tall fescue (*Festuca arundinacea* Schreb.) using the same methodology. Tall fescue injury was visually evaluated weekly until 3 WAT on a 0 (no injury) to 100 % (complete kill) scale.

Treatments were arranged in a 2 by 5 factorial randomized complete block design with three replications. ANOVA was conducted in SAS[12] with main effects and all possible interactions tested using the appropriate expected mean square values as described by McIntosh (1983). Fisher's protected LSD ($P \leq 0.05$) was used to separate means.

Dose-response experiments. Two identical experiments were initiated in 2011 in a greenhouse (Knoxville, TN; 35° 57' N Lat.) to establish does-response curves for mesotrione and topramezone with and without N. Environmental conditions in the greenhouse for the duration of these experiments are presented in Table 4.1. Smooth crabgrass[5] was seeded into 10 cm diameter pots filled with a Sequatchie loam soil [Fine-loamy, siliceous, semiactive, thermic humic Hapludult] measuring 6.2 in soil pH and 2.1% in organic matter content. This soil was blended in a 3:1 ratio with a clay mineral soil amendment[6]. Plants were watered as needed to maximize growth and vigor. Pots were hand-thinned to five smooth crabgrass plants at the 3- to 5- tiller growth stage at application. These five plants were maintained at a 7.5 cm height of cut with scissors prior to treatment.

Herbicide treatments consisted of mesotrione[1] at 0, 70, 140, 280, 560 and 1120 g ai ha^{-1} and topramezone[2] at 0, 4.5, 9, 18, 36 and 72 g ai ha^{-1}. Each herbicide included a non-ionic surfactant[3] at 0.25 % v/v. Two rates of N (0 or 49 kg ha^{-1}) were also applied forming a 6 by 2 factorial treatment arrangement. This arrangement allowed for both N-treated and N-withheld controls receiving no herbicides to be included for comparison. Immediately prior to herbicide application, N treatments were applied to each pot using 10 mL of water solution containing 8.6 g of urea (46N-0P-0K) dissolved in 1 L of water. Herbicide treatments were applied using a CO_2-powered sprayer containing four flat-fan nozzles[4] calibrated to deliver 280 L ha^{-1}. Smooth crabgrass control was visually evaluated at 4, 7, 14 and 21 DAT using a 0 (no control) to 100 % (complete kill) scale. Chlorophyll fluorescence (F_v/F_m) was also assessed[7] using methods of Elmore et al. (2011) at 4 and 7 DAT. To provide an additional quantitative measure of smooth crabgrass control, aboveground biomass was harvested from each pot 21 DAT and dried in an oven[8] at 100 °C for 4 d and weighed. Treatment means were expressed as a percent reduction in aboveground biomass of the untreated control for both N-treated and N-withheld plants individually.

The experiment was arranged in a completely randomized design with four replications. ANOVA was conducted in SAS[12] with main effects and all possible interactions tested using the appropriate expected mean square values as described by McIntosh (1983). Smooth crabgrass control data from 21 DAT were regressed over herbicide dose using a log-logistic model in Prism[13]:

$$Y = C + ([D - C) / [1 + X / I_{50})^{B}]) \qquad [1]$$

Where Y represents smooth crabgrass control, X represents the $\log_{10}$ of the herbicide dose applied, C is the lower limit for Y (i.e., 0% smooth crabgrass control), D is the upper limit for Y (i.e., 100% smooth crabgrass control), I_{50} is the herbicide dose resulting in 50% control, B is the slope of the line at I_{50}, and (Seefeldt et al. 1995). Significant differences between I_{50} values of different nitrogen levels were determined using a lack of fit F-test ($P \leq 0.05$) in Prism.

Leaf-response experiments. Research was conducted in 2011 in a greenhouse (Knoxville, TN; 35° 57' N Lat.) to determine the effects of mesotrione and topramezone on leaf tissue production and chlorophyll and carotenoid pigments when applied with and without N. Experiments were repeated in time. Environmental conditions in the greenhouse during each experimental run are listed in Table 4.1.

Smooth crabgrass was seeded into 15 cm diameter pots filled with a Sequatchie loam soil [Fine-loamy, siliceous, semiactive, thermic humic Hapludult] measuring 6.2 in soil pH and 2.1% in organic matter content. This soil was blended in a 3:1 ratio with a clay mineral soil amendment[6]. Plants were clipped weekly to a 10 cm height with scissors. Pots were hand-thinned to contain eight plants at the 3- to 5- tiller growth stage at study initiation. Prior to herbicide treatment all leaves (except the bud leaf) of each tiller were marked with a small (< 5 mm length) amount of indelible ink[9] to facilitate future separation of leaves emerged before and after herbicide treatment. Plants were treated with 0 or 49 kg N ha^{-1} and mesotrione (280 g ai ha^{-1}) or topramezone (18 g ai ha^{-1}), forming a 2 by 2 factorial treatment arrangement. Both N-treated and N-withheld controls receiving no herbicides were included for comparison. Immediately prior to herbicide application, N treatments were applied to each pot using 20 mL of water

solution consisting of 9.7 g of urea (46N-0P-0K) dissolved in 1 L of water. Herbicide treatments were applied with non-ionic surfactant[3] (0.25% v/v) and 430 L ha^{-1} of water carrier through a single flat-fan nozzle[10] in a spray chamber[11]. Irrigation was withheld for 18 h after treatment application.

Leaves were harvested for pigment quantification 10 DAT. For each pot, leaves from all plants were dissected from stem and sheath tissue and separated into two groups: leaf tissue present at herbicide application (OLD) and leaf tissue produced after herbicide application (NEW). Percent necrosis for both OLD and NEW leaves was determined visually using a 0 (no necrosis) to 100% (complete necrosis) scale. After assessing necrosis, leaf tissue was immediately frozen in liquid N and placed on ice for transfer to storage at -80°C. Prior to pigment extraction, fresh weights of OLD and NEW leaf tissues were determined. A 0.25 g fresh weight leaf tissue sample was then analyzed for carotenoid and chlorophyll pigment concentrations. Leaf tissue pigments were extracted and then quantified through HPLC (high-pressure liquid chromatography) using previously described methods (Brosnan et al. 2011; Kopsell et al. 2007). Pigments are expressed as mg 100 mg fresh weight $(FW)^{-1}$ of smooth crabgrass leaf tissue.

Pots were arranged in a completely randomized design with four replications. ANOVA was conducted in SAS[12] with main effects and all possible interactions tested using the appropriate expected mean square values as described by McIntosh (1983) Fisher's protected LSD ($P \leq 0.05$) was used to separate means.

RESULTS AND DISCUSSION

Field Experiments. Herbicide-by-year interactions were present in smooth crabgrass control data 4 and 7 DAT. However, treatment with N significantly ($P \leq 0.001$) increased smooth crabgrass control with both herbicides each year. At 4 DAT, mesotrione applied without N provided < 10% smooth crabgrass control each year, while application with N (49 kg ha^{-1}) provided 40% control in 2010 and 70% control in 2011. Responses from topramezone applications were similar. By 7 DAT, an application of 49 kg N ha^{-1} improved control of smooth crabgrass with mesotrione and topramezone by 30% each year.

No main effect interactions with year or herbicide were detected in smooth crabgrass control data collected 2 to 8 WAT; therefore, data were combined across years and herbicides (Table 4.2).

On every evaluation date all rates of N increased smooth crabgrass control with mesotrione and topramezone. While 25 kg N ha^{-1} improved smooth crabgrass control greater than 12 kg N ha^{-1} on most dates, few improvements were observed with N rates greater than 25 kg ha^{-1}. These results indicate that N application at rates as low as 12 kg ha^{-1} improve smooth crabgrass control with mesotrione and topramezone and increasing N application to 25 kg ha^{-1} will further improve control. For example, smooth crabgrass was controlled 30% 7 WAT when herbicide was applied without additional N. An application of N at 12 kg ha^{-1} improved control to 49%, while 25 kg N ha^{-1} further improved control to 67% by 7 WAT. Visual turfgrass injury ranged from 11 to 15% 14 DAT and dissipated by 21 DAT; N rate did not affect injury (data not presented).

Turfgrass managers seeking to improve smooth crabgrass control with mesotrione and topramezone should apply N at rates 12 to 25 kg ha^{-1}.

Dose-Response Experiments. Significant herbicide-by-experimental run interactions were detected in smooth crabgrass control and F_v/F_m data collected from 4 to 14 DAT; however, N and herbicide rate increased ($P < 0.001$) smooth crabgrass control and reduced aboveground biomass 4 to 14 DAT in each experimental run (data not presented). While F_v/F_m decreased with increasing herbicide rate 4 and 7 DAT, N treatment did not affect F_v/F_m (data not presented). Herbicide-by-experimental run interactions were also present in aboveground biomass data; thus, data from each run are presented separately. No herbicide-by-experimental run interactions were present in smooth crabgrass control data 21 DAT. Therefore, log-logistic regression analyses were conducted using combined data from both experimental runs.

N application decreased ($P < 0.001$) the amount of mesotrione and topramezone required to control smooth crabgrass by 50% (I_{50}) 21 DAT (Figure 4.1). Mesotrione I_{50} values were reduced more than 65% from 524 to 169 g ha^{-1} by N application. Topramezone I_{50} values were reduced more than 50% from 43.3 to 20.2 g ha^{-1} by N application. Similarly, Cathcart et al. (2004) reported N application reduced the amount of mesotrione required to decrease redroot pigweed biomass by 50% (GR_{50}) more than 70%

N application also enhanced aboveground biomass reductions with all rates of mesotrione and topramezone (Table 4.3). When applied with N in the first experimental run, mesotrione at 70 g ha^{-1} reduced aboveground biomass 61%, while mesotrione rates of 280, 560 and 1120 g ha^{-1} were required to achieve a similar reduction when N was

withheld. The 1120 g ha^{-1} rate of mesotrione reduced aboveground biomass 96% when applied with N, but only 59% when N was withheld. Similar responses were observed in the second experimental run with mesotrione. In both experimental runs, topramezone at ≥ 9 g ha^{-1} reduced aboveground biomass 75 to 94 % when applied with N compared to 17 to 62% when N was withheld. Data from dose-response experiments indicate that soil-applied N at 49 kg ha^{-1} enhances the activity of mesotrione and topramezone at a variety of herbicide rates on 3- to 5- tiller smooth crabgrass.

Leaf-Response Experiments.

Leaf number, weight and necrosis. Main effect interactions with experimental run were not significant, therefore data from each experimental run were pooled. Applied N affected smooth crabgrass growth in these studies. The number of NEW leaves averaged 106 and 178 per pot for control plants receiving 0 and 49 kg N ha^{-1}, respectively. Similarly, NEW fresh leaf weights measured 2.1 and 3.6 g for control plants receiving 0 and 49 kg N ha^{-1}, respectively. Nitrogen did not affect the number or weight of OLD leaves present on non- or herbicide-treated plants. Nitrogen had no effect on the number of NEW leaves produced after herbicide treatment as well (Table 4.4). However, N treatment did reduce the weight of NEW leaf tissue in topramezone-treated plants. Treatment with N also increased necrosis of OLD and NEW leaves following mesotrione and topramezone application. Nitrogen increased leaf necrosis of mesotrione-treated plants by 8 and 15% for OLD and NEW leaves, respectively. Similarly, N increased leaf necrosis of topramezone-treated plants by 12 and 24% for OLD and NEW leaves, respectively.

Leaf tissue chlorophyll and carotenoid concentration: As was observed by Goddard et al. (2010), mesotrione and topramezone preferentially bleached newer leaves formed after herbicide application. While a direct statistical comparison was not made, carotenoid and chlorophyll pigment concentrations in NEW leaves were lower than those in OLD leaves after treatment with mesotrione and topramezone (Table 4.5).

Nitrogen did not affect total chlorophyll, total xanthophyll or lutein concentrations in OLD leaves of herbicide-treated plants. Nitrogen decreased total chlorophyll, total xanthophyll and lutein concentrations in NEW leaf tissue in topramezone-treated plants by approximately 50%. Chlorophyll and carotenoid pigment concentrations in NEW leaves of mesotrione-treated plants were not affected by N. While not statistically significant, this trend was observed in necrosis data as N treatment increased necrosis by only 15% in topramezone-treated plants and 24% in mesotrione-treated plants. This could be attributed to greater activity of mesotrione as compared to topramezone at rates selected for this experiment. NEW leaf pigment concentrations and necrosis in N-withheld plants treated with mesotrione were similar to those of N-treated topramezone plants, suggesting herbicidal activity of these treatments were similar. Mesotrione alone may have caused near-threshold reduction in pigments, reducing the ability of N to cause further reductions. Moreover, immature (new) shoot tissues have lower pigment concentrations than fully mature shoot tissues (Lefsrud et al. 2007). Lower pigment concentrations present in immature tissues, combined with greater tissue necrosis may have resulted in data observed in pigment responses to mesotrione in the current study.

Concentrations of the carotenoid precursor phytoene in NEW leaves of herbicide-treated plants measured 0.26 mg 100g FW^{-1}. This indicates observed reductions in carotenoid pigment concentrations are likely due to a lack of phytoene desaturase, a symptom of HPPD-inhibiting herbicide application (Kopsell et al. 2010; Mayonado et al. 1989). Phytoene concentrations did not differ between mesotrione and topramezone and was not affected by N treatment. Phytoene was not detected in OLD leaves of herbicide-treated plants or in NEW or OLD leaves of non-herbicide-treated plants.

Leaf-response data indicate N does not enhance efficacy of mesotrione and topramezone by increasing bleached leaf production. Rather, carotenoid and chlorophyll pigment data suggest efficacy enhancement may be caused by an increase in the activity of these herbicides in shoot meristems. Future research should investigate whether N fertility status increases phloem translocation of these herbicides to shoot meristems due to increased photoassimilate production, as was reported by Mithila et al. (2008) with glyphosate. Increased translocation has been attributed to N-enhanced efficacy of flazasulfuron as well (Brosnan et al. 2010). Comparisons of different water-soluble N sources and timing of N application relative to herbicide treatment are also warranted and would have implications for turfgrass managers.

SOURCES OF MATERIALS

[1]Tenacity® 4 SC, Syngenta Professional Products. P.O. Box 18300, Greensboro, NC 27419.

[2]Impact® 2.8 SC, Amvac Chemical. 4100 E. Washington Blvd., Los Angeles, CA 90023.

[3]Activator-90. Loveland Products Inc. 3005 Rocky Mountain Ave., Loveland, CO 80632.

[4]Teejet 8002 flat fan spray nozzle. Spraying Systems Co. P.O. Box 7900, Wheaton, IL 10902.

[5]Smooth crabgrass (*Digitaria ischaemum*) seed. Herbiseed. New Farm/Mire La West End, Reading RG10 0NJ, U.K.

[6]Turface. Profile Products, LLC. 750 Lake Cook Rd., Buffalo Grove, IL. 60089.

[7]OS1-FL, Opti-sciences Inc. 8 Winn Ave., Hudson, NH 03051.

[8]Model LR-271C; Greive Corporation, 500 Hart Rd., Round Lake, IL 60073.

[9]Sharpie® Fine Point black marker. Newell Rubbermaid, 3 Glenlake Pkwy., Atlanta, GA. 30328.

[10]Teejet 8004EVS flat fan spray nozzle. Spraying Systems Co. P.O. Box 7900, Wheaton, IL 10902 .

[11]Generation III Research Sprayer. DeVries Manufacturing, 28081 870th Ave., Hollandale, MN 56045.

[12]Statistical Analysis Software, Inc., 100 SAS Campus Drive, Cary, NC 27513.

[13]Prism 5.0 for Mac OSX, GraphPad Software, 2236 Avenida de la Playa, LaJolla, CA. 92037.

ACKNOWLEDGEMENTS

The authors would like to thank Veronica Sublett, Jake Huffer, Daniel Farnsworth, and Shane Breeden for their assistance in conducting these experiments. Additionally, the authors would like to thank the Tennessee Agricultural Experiment Station. Mention of trade names or commercial products in this publication is solely for

the purpose of providing specific information and does not imply recommendation or endorsement by the University of Tennessee Institute of Agriculture.

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APPENDIX

TABLES AND FIGURES

Table 4.1. Environmental conditions during experiments evaluating the response of smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.) in a greenhouse in Knoxville, TN (35° 57' N Lat.) in 2011.

		Air temperature[b]			Relative humidity			PAR[a]		
Experiment	Experimental Run	Avg.	High	Low	Avg.	High	Low	Min. peak	Max. peak	Avg. 24-hr total
		°C			%			μmol m^{-2} s^{-1}		mol m^{-2} d^{-1}
Dose-Response	A	26	33	20	69	94	47	1270	1890	23.6
	B	26	36	19	81	98	45	1060	1940	19.3
Leaf-Response	A	27	33	23	79	94	49	544	1618	18.4
	B	25	33	21	76	96	44	738	1617	18.8

[a]Abbreviations: Photosynthetically active radiation, PAR.
[b]Temperature and relative humidity were recorded every five minutes and averaged for the duration of each experiment; 24-hour high and low temperatures were averaged for the duration of each experiment. Photosynthetically active radiation (PAR) values were recorded every 15 minutes. Peak PAR values were determined for each 24-hour period; the minimum and maximum of these 24-hour peak values were recorded for each experiment. 24-hour PAR totals were recorded and then averaged for the duration of each experiment.

Table 4.2. Percent control of smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.) 2 to 8 weeks after treatment (WAT) with mesotrione (280 g ha^{-1}) or topramezone (9 g ha^{-1}) in combination with nitrogen (N) at 0, 12, 25, 37 or 49 kg ha^{-1} in 2010 and 2011. Herbicides were applied with a non-ionic surfactant at 0.25% v/v. Data are combined across years and herbicides.

	Smooth crabgrass control						
Nitrogen[a]	2 WAT[b]	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
kg ha^{-1}	%						
0	55	59	53	51	47	30	39
12	73	74	65	60	58	49	50
25	86	86	82	77	69	67	67
37	91	89	84	82	69	61	61
49	86	81	79	79	67	62	56
LSD[c]	12	11	11	12	15	17	18

[a]Nitrogen was applied as granular ammonium sulfate (21-0-0) and watered in prior to herbicide application.
[b] Abbreviations: least significant difference, LSD; weeks after treatment, WAT.
[b]Means separated using Fisher's protected least significant difference test ($P \leq 0.05$).

Table 4.3. Aboveground smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.) percent biomass reduction 21 days after application of five rates of mesotrione (70, 140, 280, 560, and 1120 g ha^{-1}) and topramezone (4.5, 9, 18, 36, and 72 g ha^{-1}). Means are expressed as a percent reduction compared to N-treated- or N-withheld-control for plants treated with and without soil-applied N (49 kg ha^{-1}), respectively.

			Aboveground biomass	
Herbicide	Rate	N[a]	Run A	Run B
	—g ha^{-1}—	—kg ha^{-1}—	——% reduction——	
Mesotrione	70	0	17	15
		49	61	62
	140	0	25	38
		49	83	79
	280	0	55	47
		49	91	84
	560	0	52	57
		49	95	90
	1120	0	59	70
		49	96	89
Topramezone	4.5	0	-9	3
		49	41	37
	9	0	35	27
		49	81	75
	18	0	17	46
		49	83	80
	36	0	45	57
		49	93	87
	72	0	51	62
		49	94	87
		LSD[b]	25	19

[a]Nitrogen was applied as urea (46-0-0) solution.
[b]Means separated using Fisher's protected least significant difference test ($P \leq 0.05$).

Table 4.4. Necrosis, leaf number, and fresh weight of smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.) leaves emerged before (OLD) and 10 days after (NEW) treatment with the combination of mesotrione (280 g ha^{-1}) or topramezone (18 g ha^{-1}) and 0 or 49 kg N ha^{-1}. Means are comprised of 8 observations. Necrosis was visually evaluated on a 0 (no necrosis) to 100 (complete necrosis) percent scale.

		Leaf necrosis		Leaf number		Leaf weight	
Herbicide	N[a]	NEW[b]	OLD	NEW	OLD	NEW	OLD
	kg ha^{-1}	%		#		g	
Mesotrione	0	28	4	67	60	0.71	0.55
	49	43	12	62	55	0.59	0.43
Topramezone	0	10	4	67	53	0.84	0.53
	49	34	16	57	51	0.61	0.45
	LSD[c]	10.3	6.5	NS	NS	0.18	NS

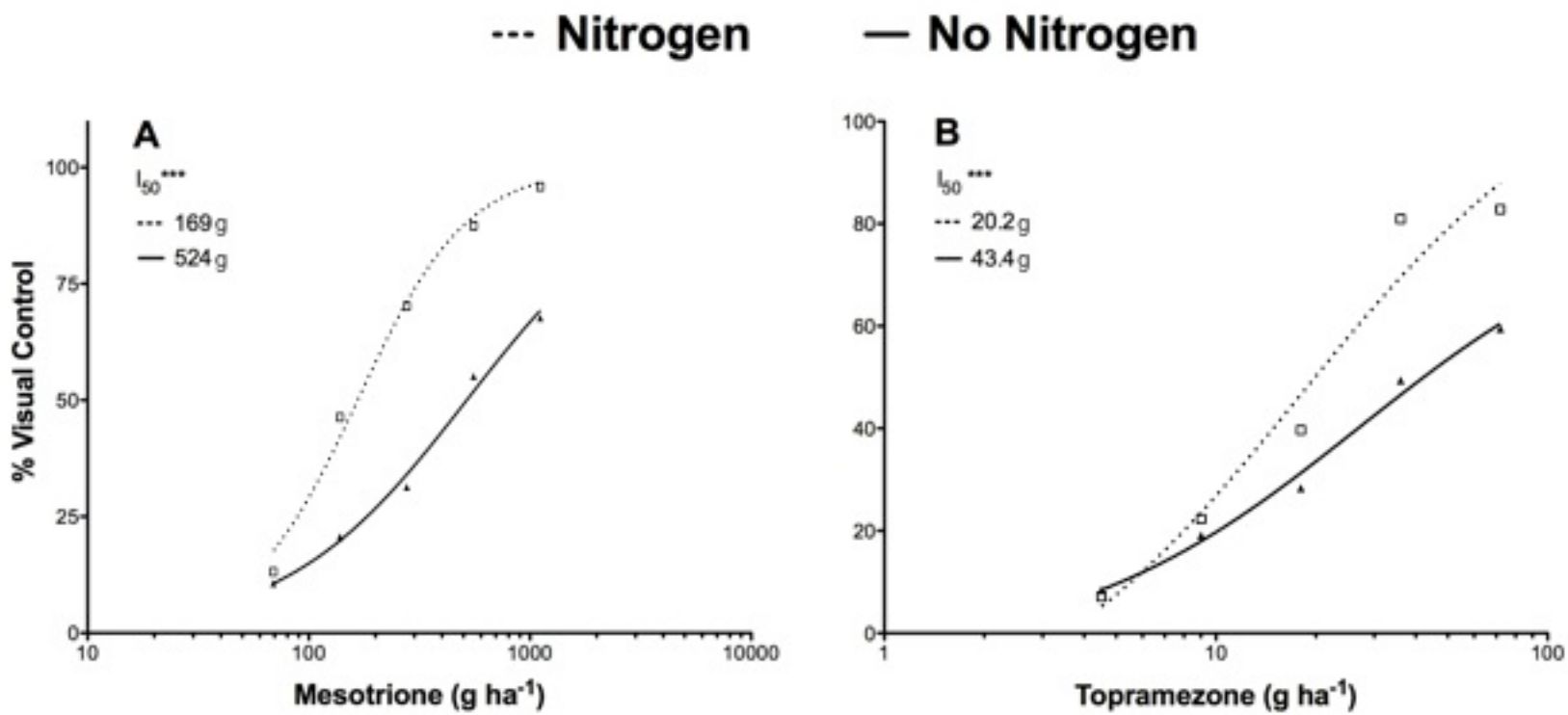
[a]Nitrogen was applied as urea (46-0-0) solution.
[b]Abbreviations: new leaf tissue after herbicide application, NEW; leaf tissue present when herbicides were applied, OLD; non-significant, NS
[c]Means separated using Fisher's protected least significant difference test ($P \leq 0.05$).

Table 4.5. Total chlorophyll (chlorophyll *a* + *b*), lutein, and total xanthophyll cycle (antheraxanthin + violaxanthin + zeaxanthin) pigment concentrations in smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.) leaves emerged before (OLD) and 10 days after (NEW) treatment with the combination of mesotrione (280 g ha^{-1}) or topramezone (18 g ha^{-1}) and 0 or 49 kg N ha^{-1}. Means are comprised of 8 observations.

		Total chlorophyll		Total xanthophyll cycle		Lutein	
	N[a]	NEW[b]	OLD	NEW	OLD	NEW	OLD
Herbicide	—kg ha^{-1}—	——mg 100 g FW^{-1}[c]——					
Mesotrione	0	27.6	130.7	2.5	9.9	2.3	2.3
	49	22.9	126.7	1.9	8.5	2.0	2.0
Topramezone	0	48.0	144.7	4.6	9.7	3.9	3.9
	49	24.9	138.2	2.3	8.3	2.0	2.0
	LSD[c]	11.9	NS	1.2	NS	0.9	NS

[a]Nitrogen was applied as urea (46-0-0) solution
[b]Abbreviations: new leaf tissue after herbicide application, NEW; leaf tissue present when herbicides were applied, OLD; non-significant, NS; fresh weight, FW
[c]Means separated using Fisher's protected least significant difference test ($P \leq 0.05$)
[c]Pigment concentrations expressed as mg 100 g fresh weight^{-1} (FW) of smooth crabgrass leaf tissue

Figure 4.1. Control of smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.) in a greenhouse following applications of mesotrione (A) (70, 140, 280, 560, and 1120 g ha^{-1}) topramezone (B) (4.5, 9, 18, 36, and 72 g ha^{-1}) with and without soil-applied urea (49 kg N ha^{-1}) 21 days after treatment. I_{50} is the rate of herbicide (g ha^{-1}) that controlled smooth crabgrass by 50%. I_{50} values were compared with an F-test. *** indicates significance at the $P \leq 0.001$ level. Data were combined across two experimental runs.



CHAPTER 4

GROWING DEGREE-DAY-BASED APPLICATION TIMINGS AFFECT DALLISGRASS (*PASPALUM DILATATUM*) CONTROL IN TALL FESCUE.

This chapter is based on a paper intended for publication by Matthew Elmore, James Brosnan, Thomas Mueller, Brandon Horvath, Dean Kopsell and Gregory Breeden:

Elmore, M.T., J.T. Brosnan, T.C. Mueller, B.J. Horvath, D.A. Kopsell, and G.K. Breeden. 2011. Growing Degree-Day-Based Application Timings Affect Dallisgrass (*Paspalum dilatatum*) Control in Tall Fescue. Weed Technology (To be submitted: October 2012).

My primary contributions to this paper include (i) Design and conducting the experiments, (ii) processing, analyzing and interpreting data, (iii) reading literature, (iv) writing the manuscript.

ABSTRACT

Control of perennial grassy weeds such as dallisgrass (*Paspalum dilatatum* Poir.) can vary by seasonal application timing. Field research was conducted in 2010 and 2011 investigating the efficacy of herbicides for dallisgrass control when applied at various growing (GDD) or cooling degree-day (CDD)-based application timings. Herbicides included fluazifop (105 g ha^{-1}) alone or in combination with mesotrione (280 g ha^{-1}), tembotrione (92 g ha^{-1}), or topramezone (27 g ha^{-1}). Herbicide treatments were applied at 75, 175, 375, 775 GDD, or 5 CDD. Treated plots were subjected to three tall fescue seeding regimes: no seeding, seeding in spring or seeding in fall. In 2010, dallisgrass control when herbicide treatments were applied at 75, 375 and 775 GDD was poor (< 60%) by 52 weeks after treatment (WAT). Tall fescue injury resulting with fluazifop-

containing treatments applied at 375 and 775 GDD was unacceptable (> 20%) in both 2010 and 2011. When applied at 175 GDD or 5 CDD in 2010, dallisgrass control ranged from 76 to 89%, respectively at 52 WAT. The addition of mesotrione, tembotrione or topramezone to fluazifop did not affect dallisgrass control at any application timing. Interseeding tall fescue in the fall improved dallisgrass control from herbicides applied at 175, 375 and 775 GDD when assessed 52 WAT. Results from 2010 indicate that dallisgrass is most susceptible to fluazifop applications at 175 GDD and 5 CDD and that fall seeding can improve control with herbicide treatment at 175 GDD. Future research should evaluate dallisgrass control programs involving sequential applications of fluazifop (105 g ha^{-1}) at 175 GDD and 5 CDD combined with a fall seeding.

INTRODUCTION

Dallisgrass (*Paspalum dilatatum* Poir.) is a problematic warm-season perennial weed in the mid-Atlantic and southeastern regions of the United States (Beard 2002). Compared to tall fescue (*Festuca arundinacea* Schreb.), dallisgrass possess a lighter green color, coarser texture and faster growth rate during the warmer months, reducing the aesthetic and functional quality of the turfgrass sward (Elmore and Cudney 2001). Tolerance to close mowing, traffic, and high soil moisture enhance dallisgrass persistence in turfgrass stands (Henry et al. 2008; Henry et al. 2009; Rubio et al. 1995; Striker et al. 2006).

Options for selective dallisgrass control in tall fescue are limited. Monosodium methanearsonate (MSMA) is labeled for use in tall fescue, although turfgrass injury is common (Anonymous 2009; Johnson 1997). EPA restrictions currently prohibit use of MSMA on athletic field and residential turf areas with use on sod farms and golf courses scheduled to be prohibited after 2013 (United States EPA 2009). The 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor mesotrione is labeled for use in tall fescue and has activity against dallisgrass, but long-term control following treatment at 280 g ha^{-1} has been negligible (Reicher et al. 2005). Topramezone and tembotrione are HPPD-inhibitors that have greater efficacy than mesotrione against various grassy weeds in row crop production (Bollman et al. 2008). However, data evaluating the use of these HPPD-inhibitors for dallisgrass control in turf are limited. The acetyl-CoA carboxylase (ACCase) inhibitor fluazifop-p-butyl (hereafter referred to as fluazifop) has efficacy against dallisgrass in pastures at rates $\geq$ 140 g ha^{-1}, but provides almost no control 1 year after treatment (Evers et al. 2002).

Cultural practices promoting healthy turfgrass help eliminate voids in the canopy (Turgeon 2005). Eliminating these voids with desirable turfgrass species reduces niches for weed sustenance (Watschke and Engel 1994). Effects of overseeding on weed control in turfgrass are varied. Without chemical or biological weed control, several researchers have reported overseeding has no effect on weed populations (Elford et al. 2008; Larsen et al. 2004; Larsen and Fischer 2005). However, Kopec and Gilbert (1999) reported perennial ryegrass overseeding in combination with sulfentrazone resulted in less annual bluegrass infestation than sulfentrazone alone. In another study, the combination of overseeding and a fungal control agent reduced dandelion and white clover (*Trifolium repens* L.) populations more than overseeding or the fungal control agent applied alone (Abu-Dieyeh and Watson 2007). These studies suggest the combination of overseeding and herbicides may provide greater dallisgrass control than overseeding or herbicides applied alone; however, this has not been evaluated.

Growing degree-day (GDD) accumulation is commonly used to quantify the influence of air temperature on plant development throughout a growing season (McMaster and Wilhelm 1997). In turfgrass, GDD accumulation can predict annual bluegrass (*Poa annua*) and Kentucky bluegrass (*Poa pratensis* L.) seedhead emergence, smooth crabgrass (*Digitaria ischaemum* (Schreb.) ex Muhl.) emergence, Kentucky bluegrass root formation and viable creeping bentgrass (*Agrostis stolonifera* L.) root length (Danneberger and Vargas 1984; Fidanza et al. 1996; Koski et al. 1988; Schlossberg and Karnok 2002). Accurately determining the occurrence of these phenological events allows annual bluegrass seedhead suppressants, PRE crabgrass control herbicides, and growth regulators to be applied using GDD-based application

timings to maximize efficacy, rather than using a calendar date. (Branham and Danneberger 1989; Fidanza et al. 1996; Kreuser and Soldat 2011). In studies evaluating optimum GDD-based 2,4-D application timing for dandelion (*Taraxacum officinale* F.H. Wigg.) control, Schleicher et al. (1995, 1996) determined that the efficacy of ester and amine formulations of 2,4-D + 2,4-DP against dandelion improved when applied after 130 to 145 GDD_{10C}, which they attributed to improved 2,4-D phloem translocation. Investigating dallisgrass control involving GDD-based application timings, Brosnan et al. (2010a) reported > 90% control of dallisgrass 76 days after initial treatment (DAIT) from single and sequential applications of fluazifop (105 g ha^{-1}) made in early spring (< 160 GDD_{10C}) but < 40% control from applications in early summer (> 500 GDD_{10C}). Investigating mixtures of fluazifop + triclopyr for bermudagrass (*Cynodon dactylon* (L.) Pers.) suppression, Brosnan et al. (2011) determined applications in early spring (200 GDD_{10C}) and late summer (2250 GDD_{10C}) were more effective than late-spring and mid-summer applications. Given the seasonal variability in dallisgrass control with fluazifop, a more complete investigation to determine the optimum GDD-based application timing is warranted.

The objective of this research was to evaluate the seasonal variability in dallisgrass control provided by fluazifop alone or in combination with HPPD-inhibiting herbicides and tall fescue overseeding.

MATERIALS AND METHODS

Field site. Experiments were initiated in 2010 and 2011 on a field site of 'Coyote II' and 'Kentucky 31' tall fescue at the East Tennessee Research and Education Center

(Knoxville, TN; 35° 57' N Lat.) naturally infested with dallisgrass. Soil was a Sequatchie loam [Fine-loamy, siliceous, semiactive, thermic humic Hapludult] measuring 6.5 in soil pH and 3.8% in organic matter content. Experiments were conducted in an area of full sunlight, mowed twice weekly at 7.5 cm and irrigated as needed to promote active growth. In 2009, prior to experiment initiation, the site was slit-seeded with 'Coyote II' tall fescue at 353 kg of pure live seed (PLS) ha^{-1}.

Treatment application. Eight treatments were evaluated in 2010 and 2011: (1) fluazifop[1] (105 g ai ha^{-1}); (2) mesotrione[2] (280 g ai ha^{-1}); (3) topramezone[3] (27 g ai ha^{-1}); (4) tembotrione[4] (92 g ai ha^{-1}); (5) fluazifop + mesotrione; (6) fluazifop + topramezone; (7) fluazifop + tembotrione. An untreated control was included for comparison. A non-ionic surfactant[5] was included with all herbicide treatments at 0.25% v/v. Herbicide treatments were applied using a CO_2-powered sprayer containing four flat-fan nozzles[6] calibrated to deliver 280 L ha^{-1}.

Herbicide treatments were applied singly at five GDD- or cooling degree day (CDD)-based application timings: 75, 175, 375, 775 GDD, or 5 CDD. Growing- and cooling-degree-day accumulation was calculated using Equation 1 and Equation 2, respectively.

$$GDD = [(T_{max} - T_{min})/2] - T_{base} \qquad [1]$$

$$CDD = T_{base} - [(T_{max} - T_{min})/2] \qquad [2]$$

In these equations, T_{max} represents the daily maximum air temperature, T_{min} represents the daily minimum air temperature and T_{base} is the lowest temperature at which the biological process of interest (e.g., plant growth) does not advance (McMaster and Wilhem 1997). Similar to Brosnan et al. (2010, 2011) T_{base} used for GDD equations was

10 C. Considering that CDD accumulation was a function of when average daily temperatures fell below a certain threshold, T_{base} was 21 C in Equation 2. This value was selected as daily increases in dallisgrass shoot weight at 21 C can be nearly 50% of those in optimal temperatures (Mitchell 1956). Brosnan et al. (2011) suggested using a cooling accumulation model to schedule fall herbicide applications for perennial grassy weed control might be preferred over GDD accumulation model. Additionally, average daily air temperatures below 21 C usually indicate a seasonal shift in Knoxville, TN based on a 30-year climatological average. The use of CDD accumulation to estimate plant growth or schedule fall herbicide applications has not been examined. GDD accumulation began on January 1 and continued until July 1. CDD accumulation began on July 1 and continued to December 31. Air temperature was measured using a weather station[7] located approximately 200 yards from the field site. Daily GDD and CDD accumulation in 2010 and 2011 are plotted in Figure 5.1 and compared to 30-year averages for Knoxville, TN. Calendar dates for each herbicide application in this study are listed in Table 5.1.

All plots were subjected to three seeding regimes: no seeding, spring seeding at 353 kg PLS ha^{-1}, or fall seeding at 353 kg PLS ha^{-1}. Spring seeding treatments were applied two weeks after 175 GDD herbicide treatments were applied (May 6, 2010 and April 28, 2011), while fall seeding treatments were applied two weeks after the 5 CDD herbicide treatments were applied (September 22, 2010 and September 23, 2011) each year. 'Coyote II' tall fescue was used in 2010 and 'Falcon IV' tall fescue was used in 2011. While using different tall fescue genotypes is not ideal, 'Falcon IV' and 'Coyote II' performed similarly in trials conducted by the National Turfgrass Evaluation Program in

categories of percent establishment, weed infestation, quality, spring, summer, and fall density, brown patch (causal agent: *Rhizoctonia solani* Kühn) and *pythium* blight (causal agent: *Pythium* spp.) incidence; however, 'Falcon IV' had slightly superior transition zone quality and seedling vigor (NTEP 2005). Seeding treatments were applied using a slit-seeder[8]. A complete (24N-6P_2O_5-12K_2O) fertilizer[9] at 49 kg N ha^{-1} was broadcast over the entire experimental area immediately after seeding and shortly after spring greenup the following year (March 3, 2011 and March XX 2012)

Treatment evaluation and statistical analysis. Treatments were arranged in a split-split plot design with three replications. Application timing served as the whole plot treatment, herbicide served as the subplot treatment, and seeding served as the sub-subplot treatment. Whole plots measured 12.2 x 5.5 m; sub-plots measured 1.5 x 5.5 m; sub-sub-plots measured 1.5 x 1.8 m. In order to characterize dallisgrass morphology at each application timing, 20 dallisgrass plants were randomly selected from each subplot immediately before applying herbicide treatments. The diameter (distance across the base between the inner-most ring of leaves) and number of leaves were recorded for each plant (Table 5.2).

Dallisgrass control in each whole- and subplot was visually evaluated 2, 4, 6, 8, 18 weeks after herbicide treatment (WAT) on a 0 (no control) to 100 % (complete kill) scale. At 52 WAT, dallisgrass control was visually evaluated in all whole, sub and sub-sub plots using the aforementioned percent scale. Additionally, dallisgrass control was assessed quantitatively using a 100 x 100 cm grid containing 81 squares (10 x 10 cm). The presence or absence of dallisgrass was noted in each square. Grid counts were correlated with visual assessments of dallisgrass control 52 WAT. Tall fescue injury was

visually evaluated in both whole and subplots at 1, 2 and 4 WAT using a 0 (no injury) to 100 % (complete death) scale as well.

All data were subjected to ANOVA in SAS[11] ($P < 0.05$) using the appropriate expected mean square values as described by McIntosh (1983). Dalligrass control and grid count data 52 WAT in 2011 were not available as of this writing. Thus, these data were excluded from analysis and are not presented. Pairwise contrasts were used to evaluate effects of fluazifop-containing treatments compared to non-fluazifop containing treatments at each application timing. Significant year-by-treatment interactions were detected in dallisgrass control data; therefore, data from each year were analyzed and are presented individually.

RESULTS AND DISCUSSION

Dallisgrass control. Significant application timing-by-herbicide interactions were detected on every evaluation date in 2010. In 2011, significant application timing-by-herbicide interactions were only detected 2 WAT. Dallisgrass control varied due to herbicide treatment on every evaluation date each year. Dallisgrass diameter or number of leaves did not explain control in 2010 or 2011 (Table 5.2).

Initial dallisgrass control (2 to 8 WAT). Dallisgrass control was similar for fluazifop treatments applied at 175, 375, 775 GDD and 5 CDD at 2 WAT; dallisgrass control ranged from 40 to 87 % in 2010 and 30 to 65% in 2011 (Data 5.1). Comparatively, dallisgrass control with mesotrione, tembotrione, and topramezone ranged from 10 to 62% and 3 to 58% by 2 WAT in 2010 and 2011, respectively. Instances of fluazifop +

HPPD-inhibiting herbicide combinations reducing dallisgrass control compared to fluazifop alone were few and transient, suggesting HPPD-inhibiting herbicides do not antagonize fluazifop against dallisgrass. When applied at 75 GDD, fluazifop-containing treatments provided < 5% dallisgrass control 2 WAT. Reduced dallisgrass control at 75 GDD was likely related to air temperature at application, as daily low air temperatures averaged 5 C in the 7 days following the 75 GDD application each year. By 4 WAT, few differences in dallisgrass control were observed between fluazifop-containing treatments regardless of application timing in 2010 and 2011, as control ranged from 60 to 98% at all timings. When evaluated 8 WAT, fluazifop-containing treatments applied at 175 GDD and 5 CDD provided greater control than those applied at 775 GDD in 2010. Dallisgrass control from fluazifop applied at 175 GDD in this study was similar to that reported by Brosnan et al. (2010) with fluazifop application at < 160 GDD_{10C}. Pairwise contrasts indicate fluazifop-containing treatments provided greater dallisgrass control than non-fluazifop-containing treatments on nearly every rating date, regardless of application timing.

Long-term dallisgrass control (18 to 52 WAT). By 18 WAT, all fluazifop-containing treatments applied at 5 CDD in 2010 provided 97 to 98% control, which was greater than control provided by the same treatments applied at 75, 375 and 775 GDD. With the exception of fluazifop + tembotrione, fluazifop-containing treatments applied at 175 GDD provided between 73 and 85% control 18 WAT. Comparatively, dallisgrass control ranged from 12 to 47% when these treatments were applied at 75, 375 and 775 GDD in 2010. Similar responses were observed in 2011 but data for the 375, 775 and 5 CDD application timings are unavailable as of this writing. The addition of mesotrione,

tembotrione, or topramezone to fluazifop did not increase dallisgrass control by 18 WAT regardless of application timing in either year.

By 52 WAT, dallisgrass control provided by herbicide applications made at 75, 375 and 775 GDD was poor (< 60%), with the exception of fluazifop + tembotrione applied at 75 GDD (74%). Fluazifop-containing treatments applied at 175 GDD and 5 CDD provided the most dallisgrass control in this study (76 to 89%) 52 WAT in 2010. Grid count data supported visual assessments of dallisgrass control 52 WAT in 2010 ($r = -0.77$; $p < 0.05$). Fluazifop-containing treatments applied at 175 and 5 CDD had ≤ 14 dallisgrass plants per plot compared to 36 plants for the untreated control. Pairwise contrasts indicate fluazifop-containing treatments provided greater dallisgrass control than non-fluazifop-containing treatments for applications at 75, 175 GDD and 5 CDD.

Tall fescue injury. Similar to responses in dallisgrass control data, significant application timing-by-herbicide interactions were detected in tall fescue injury data collected in 2010. In 2011, significant application timing-by-herbicide interactions were detected 1 and 2 WAT as well.

Tall fescue injury with mesotrione, tembotrione, and topramezone was observed 2 and 4 WAT when applied 775 GDD in 2010 (Data 5.2). In 2010, tall fescue injury from mesotrione, tembotrione, and topramezone applied at 775 GDD ranged from 5 to 33% by 4 WAT. In 2011, ≤ 10% injury was observed from mesotrione, tembotrione and topramezone on all evaluation dates regardless of application timing. Significant tall fescue injury was not observed with mesotrione, topramezone, or tembotrione applications at 75, 175, 375, or 5 CDD applications in either year. Tall fescue injury in these experiments was similar to previous reports documenting the safety of mesotrione,

tembotrione and topramezone to cool-season turfgrasses (Reis et al. 2010; Willis and Askew 2008; Willis et al. 2006).

Tall fescue injury from fluazifop-containing treatments exceeded non-fluazifop treatments on all but one evaluation date for treatments applied at 375 and 775 GDD in both years. By 4 WAT, tall fescue injury from fluazifop-containing treatments applied at 775 GDD ranged from 30 to 75% in 2010 and 12 to 20% in 2011. Comparatively, injury from fluazifop-containing treatments applied at 175 GDD was ≤ 5% 2 WAT in 2010 but > 17% by 2 WAT in 2011. Reasons for this increased tall fescue injury are unclear as daily maximum air temperatures were similar during the two weeks following the 175 GDD application each year. Injury from fluazifop-containing treatments applied at 75 GDD never exceeded 4% in 2010 and ranged from 11 to 17% by 4 WAT in 2011. No injury was observed with any herbicide treatment applied at 5 CDD in 2010. Data indicate fluazifop applications at ≥ 375 GDD will cause unacceptable (> 20%) tall fescue injury. Mesotrione, tembotrione and topramezone may also cause unacceptable injury when applied at > 775 GDD.

Seeding treatment. Significant application timing-by-seeding interactions were detected in dallisgrass control and grid count data collected 52 WAT.

In 2010, fall seeding increased dallisgrass control following herbicide treatment at 175, 375 and 775 GDD by > 15% (Table 5.3). Larger increases in dallisgrass control due to seeding were observed at the 375 and 775 GDD timings, however, herbicidal control was lower at these timings. Kopec and Gilbert (1999) reported perennial ryegrass (*Lolium perenne* L.) overseeding in combination with herbicide treatment decreased annual bluegrass invasion. Anecdotal observations suggest the fungal disease brown patch

decreased stand density of tall fescue during summer of 2011 and could explain why fall seeding did not improve control from herbicide treatments applied at 5 CDD. Survival of tall fescue seeded in spring was minimal and did not improve control at any herbicide application timing. Applications of preventative fungicides with efficacy for brown patch control may have prevented summer reductions in tall fescue stand density; however, tall fescue stand density was not measured in this research.

Conclusions. Data indicate that dallisgrass is most susceptible to fluazifop applications at 175 GDD or 5 CDD. Results are similar to those of Brosnan et al. (2010), who suggested fluazifop applications in early spring (< 160 GDD_{10C}) were more successful than those in late spring (> 500 GDD_{10C}). Seeding tall fescue in the fall improved dallsigrass control with 175 GDD applications of fluazfop. Future research should evaluate dallisgrass control programs involving sequential applications of fluazifop (105 g ha^{-1}) at 175 GDD and 5 CDD combined with a fall seeding to provide end-users with improved strategies for dallisgrass control in tall fescue

Additionally, research should investigate the physiological basis for seasonal variability in dallisgrass control with fluazifop. Though no investigations on the effects of temperature on metabolism of ACCase-inhibiting herbicides were conducted in this study, more rapid fluazifop metabolism as temperatures increase in the spring could explain lack of efficacy at the 375 and 775 GDD timings. Lack of fluazifop translocation could explain low fluazifop efficacy with 75 GDD applications, as compared to the 175 GDD application. Schleicher et al. (1996) reported greater phloem translocation of 2,4-D in dandelion when it was applied at 160 GDD_{10C} than at 110 GDD_{10C}. Additionally, dallisgrass emergence from winter dormancy could explain the success of fluazifop

application at 175 GDD. Fluazifop is phloem-translocated to apical meristems, where it is most effective (Carr et al., 1986; Senseman, 2007). Leaf tissue production via apical meristems is integral to dallisgrass emergence from dormancy. Fluazifop-induced apical meristem dysfunction may be more detrimental during this emergence from winter dormancy (when few photosynthetically active leaves exist) than at other times of the year.

SOURCES OF MATERIALS

[1]Fusilade II® 2 EC, Syngenta Professional Products. P.O. Box 18300, Greensboro, NC 27419.

[2]Tenacity® 4 SC, Syngenta Professional Products. P.O. Box 18300, Greensboro, NC 27419.

[3]Impact® 2.8 SC, Amvac Chemical. 4100 E. Washington Blvd., Los Angeles, CA 90023.

[4]Laudis® 3.5 SC, Bayer CropScience LP. P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, N.C. 27709.

[5]Activator-90. Loveland Products Inc. 3005 Rocky Mountain Ave., Loveland, CO 80632.

[6]Teejet 8002 flat fan spray nozzle. Spraying Systems Co. P.O. Box 7900, Wheaton, IL 10902.

[7]HOBO U30. Onset Computer Corp. 470 MacArthur Blvd, Bourne, MA 02532.

[8]Ryan® Mataway® overseeder/dethatcher. Schiller Grounds Care Inc. One Bob-Cat Lane, P.O. Box 469, Johnson Creek, WI 53038.

[9]Complete fertilizer. Harrell's LLC. 720 Kraft Rd., Lakeland, FL. 33815.

[10]SAS Inc., 100 SAS Campus Drive, Cary, NC 27513.

ACKNOWLEDGEMENTS

The authors would like to thank Jake Huffer, Daniel Farnsworth, Shane Breeden and Veronica Sublett for their assistance in conducting these experiments. Additionally, the authors would like to thank the Tennessee Agricultural Experiment Station. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of Tennessee Institute of Agriculture.

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APPENDIX

TABLES AND FIGURES

Table 5.1. Calendar dates corresponding to herbicide applications at various growing- and cooling-degree day-based timings in 2010 and 2011.

	Calendar Date	
Application Timing	2010	2011
75 GDD[a]	April 7	March 22
175 GDD	April 22	April 14
375 GDD	May 18	May 12
775 GDD	June 15	June 13
5 CDD[a]	September 8	September 9

[a]Abbreviations: GDD, Growing-degree-day; CDD, Cooling-degree-day.

Table 5.2. Dallisgrass (*Paspalum dilatatum* Poir.) diameter (measured from the base of the inner-most ring of leaves) and number of leaves present when herbicide treatments were applied 2010 and 2011. Values reported are means comprised of measurements of 20 plants in each whole plot (12.2 x 5.5 m).

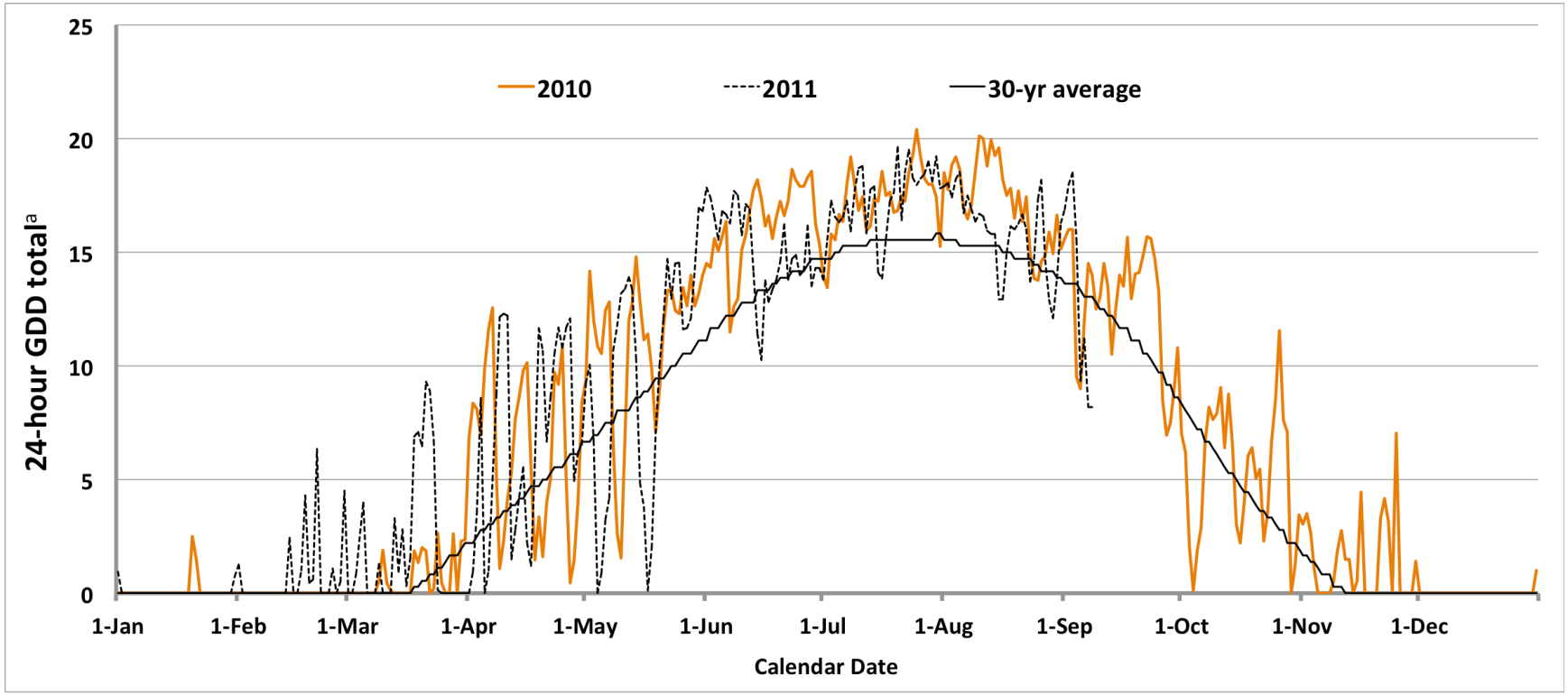
	Dallisgrass diameter		Dallisgrass leaves	
Application Timing	2010	2011	2010	2011
	cm		#	
75 GDD	2.5	2.7	36	15
175 GDD	1.7	3.1	7	17
375 GDD	2.2	3.3	11	22
775 GDD	2.1	3.7	20	41
5 CDD	2.2	3.4	44	17
LSD[a]	NS	NS	NS	6

[a]Abbreviations: Fisher's least significant difference, LSD; NS, non-significant

Table 5.3: Effect of herbicide application timing (75, 175, 375, and 775 growing degree-days and 5 cooling degree-days) and seeding treatment (no seeding, 391 kg ha^{-1} in either fall or spring) on dallisgrass (*Paspalum dilatatum* Poir.) control and grid counts 52 weeks after herbicide treatment.

		Dallisgrass	
Herbicide application timing	Seeding treatment	Control	Count
		—%—	—#[b]—
75 GDD	Fall[c]	45	27
	None	38	29
	Spring	38	23
175 GDD	Fall	73	17
	None	50	22
	Spring	54	21
375 GDD	Fall	55	31
	None	17	32
	Spring	23	36
775 GDD	Fall	65	27
	None	26	39
	Spring	35	36
5 CDD	Fall	48	33
	None	51	20
	Spring	47	24
	LSD[a]	22	17

[a]Abbreviations: Fisher's least significant difference, LSD.
[b] The number of 10 X 10 cm squares containing dallisgrass tissue in a grid containing 81 total squares.
[c] Spring seeding treatments were applied April 29 and April 21 in 2010 and 2011, respectively. Fall seeding treatments were applied September 15 and September 16 in 2010 and 2011, respectively.



[a] GDD's were calculated using the following equation: GDD = $[(T_{max} - T_{min})/2] - 10$, where T_{max} is the daily maximum air temperature, T_{min} is the daily minimum air temperature in degrees Celsius.

Figure 5.1. Twenty-four hour growing degree-day (GDD) totals in 2010 and 2011 calculated using air temperature at the East Tennessee Research and Education Center (Knoxville, TN) and a 30-year average measured at the McGee-Tyson Airport (Knoxville, TN).

Data 5.1: Dallisgrass (*Paspalum dilatatum* Poir.) control 2, 4, 8, 18 and 52 weeks after herbicide treatment (WAT) in 2010 and 2011. Control was rated on a 0 (no control) to 100 (complete kill) percent scale. Quantitative grid counts 52 WAT are also presented. Fluazifop-butyl (fluazifop), mesotrione, tembotrione and topramezone were applied at 105, 280, 92, and 25 g ha^{-1}, respectively. Herbicide treatments were applied at one of 5 application timings (75, 175, 375, and 775 growing degree-days and 5 cooling degree-days). Data from 2011 are incomplete as the experiment is still ongoing. Pairwise contrasts were used to compare effects of fluazifop containing treatments to those that did not containing fluazifop.

		Dallisgrass control									
		2 WAT		4 WAT		8 WAT		18 WAT		52 WAT	
Application timing	Herbicide treatment[a]	2010	2011	2010	2011	2010	2011	2010	2011	2010	
		%									#[c]
75 GDD	Fluazifop	0	5	72	53	55	67	28	55	39	17
	Fluazifop + mesotrione	0	2	78	67	57	73	12	78	45	26
	Fluazifop + tembotrione	3	3	67	63	87	78	37	68	74	15
	Fluazifop + topramezone	1	5	83	68	77	82	47	58	54	21
	Mesotrione	0	2	0	0	0	0	0	0	33	27
	Tembotrione	2	2	0	17	0	0	0	17	26	36
	Topramezone	7	0	0	10	0	15	0	33	29	34
	Fluazifop vs. no fluazifop	NS[d]	NS	***	***	***	***	**	***	**	**

175 GDD	Fluazifop	68	47	90	92	92	77	83	60	79	14
	Fluazifop + mesotrione	80	47	90	95	92	92	73	82	78	7
	Fluazifop + tembotrione	87	47	93	95	88	87	33	80	77	13
	Fluazifop + topramezone	80	37	92	93	93	88	85	72	89	4
	Mesotrione	25	5	10	0	0	0	20	0	32	44
	Tembotrione	43	40	20	8	52	10	50	0	54	17
	Topramezone	12	8	10	32	47	13	62	53	51	21
	Fluazifop vs. no fluazifop	***	***	***	***	***	***	NS	***	***	***
375 GDD	Fluazifop	67	62	75	85	65	67	25	.	49	24
	Fluazifop + mesotrione	63	65	78	83	50	60	27	.	28	35
	Fluazifop + tembotrione	65	55	77	83	65	67	10	.	31	41
	Fluazifop + topramezone	70	63	87	90	82	72	13	.	31	33
	Mesotrione	10	3	3	13	0	22	20	.	19	36
	Tembotrione	38	53	28	22	40	27	0	.	37	36
	Topramezone	33	47	30	38	52	37	42	.	32	30
	Fluazifop vs. no fluazifop	***	***	***	***	***	**	NS	.	NS	**

775 GDD	Fluazifop	62	57	60	90	38	60	20	.	33	37
	Fluazifop + mesotrione	40	50	60	87	32	47	0	.	29	39
	Fluazifop + tembotrione	72	40	83	92	33	73	27	.	58	24
	Fluazifop + topramezone	61	42	75	87	20	55	13	.	51	40
	Mesotrione	27	0	33	0	8	0	0	.	44	32
	Tembotrione	32	42	23	57	8	33	0	.	41	31
	Topramezone	53	58	57	55	8	0	17	.	51	33
	Fluazifop vs. no fluazifop	***	***	*	***	NS	**	NS	.	NS	**
5 CDD	Fluazifop	78	.	97	.	96	.	99	.	88	4
	Fluazifop + mesotrione	65	.	98	.	95	.	98	.	81	9
	Fluazifop + tembotrione	45	.	97	.	97	.	97	.	86	7
	Fluazifop + topramezone	50	.	98	.	98	.	98	.	76	8
	Mesotrione	13	.	10	.	18	.	0	.	17	44
	Tembotrione	58	.	42	.	32	.	20	.	6	47
	Topramezone	62	.	57	.	25	.	37	.	23	44
	Fluazifop vs. no fluazifop	*	.	***	.	***	.	***	.	***	***

LSD[b]	23	6	27	25	35	30	35	32	10	16

[a] All herbicide treatments applied with a non-ionic surfactant at a 0.25% v/v ratio
[b] Abbreviations: Days after treatment, DAT; Fisher's least significant difference, LSD.
[c] The number of 10 X 10 cm squares containing dallisgrass tissue in a grid containing 81 total squares.
[d] NS, non-significant; *, **, ***, significant at the 0.05, 0.01 and 0.001 levels, respectively

Data 5.2: Tall fescue (*Festuca arundinacea* Schreb.) injury 1, 2, and 4 weeks after herbicide treatment (WAT) in 2010 and 2011. Fluazifop-butyl (fluazifop), mesotrione, tembotrione and topramezone were applied at 105, 280, 92, and 25 g ha^{-1}, respectively. Herbicide treatments were applied at one of 5 application timings (75, 175, 375, and 775 growing degree-days and 5 cooling degree-days). Data from 2011 are incomplete as the experiment is still ongoing. Pairwise contrasts were used to compare effects of fluazifop containing treatments to those that did not containing fluazifop.

		Tall fescue injury					
		1 WAT		2 WAT		4 WAT	
Application timing	Herbicide treatment[a]	2010	2011	2010	2011	2010	2011
		%					
75 GDD	Fluazifop	3	0	0	0	0	17
	Fluazifop + mesotrione	3	0	0	0	0	13
	Fluazifop + tembotrione	4	0	0	0	0	12
	Fluazifop + topramezone	3	0	2	0	0	11
	Mesotrione	0	0	2	2	0	0
	Tembotrione	0	0	0	0	0	0
	Topramezone	0	0	0	0	0	0
	Fluazifop vs. no fluazifop	**[c]	NS	NS	NS	NS	***
175 GDD	Fluazifop	2	7	3	18	0	10
	Fluazifop + mesotrione	2	2	5	20	0	10
	Fluazifop + tembotrione	5	2	3	17	2	10
	Fluazifop + topramezone	5	5	5	17	0	10
	Mesotrione	3	0	0	0	0	0
	Tembotrione	0	0	0	0	0	0

	Topramezone	2	2	0	0	0	0
	Fluazifop vs. no fluazifop	NS	*	**	***	NS	.
375 GDD	Fluazifop	5	6	17	25	8	13
	Fluazifop + mesotrione	5	5	20	23	15	13
	Fluazifop + tembotrione	5	6	18	23	13	13
	Fluazifop + topramezone	5	5	22	25	23	17
	Mesotrione	0	0	0	0	0	0
	Tembotrione	0	0	0	0	2	0
	Topramezone	0	0	0	0	3	0
	Fluazifop vs. no fluazifop	***	***	***	***	***	***
775 GDD	Fluazifop	0	0	13	20	38	12
	Fluazifop + mesotrione	8	5	15	20	75	15
	Fluazifop + tembotrione	10	3	5	20	30	20
	Fluazifop + topramezone	7	3	7	22	58	20
	Mesotrione	10	2	7	3	5	0
	Tembotrione	0	0	5	0	18	0
	Topramezone	0	0	7	0	33	5
	Fluazifop vs. no fluazifop	**	**	NS	***	*	**
5 CDD	Fluazifop	0	.	0	.	0	.
	Fluazifop + mesotrione	0	.	0	.	0	.
	Fluazifop + tembotrione	0	.	0	.	0	.
	Fluazifop + topramezone	0	.	0	.	0	.
	Mesotrione	0	.	0	.	0	.
	Tembotrione	0	.	0	.	0	.

Topramezone	0	.	0	.	0	.
Fluazifop vs. no fluazifop	NS	.	NS	.	NS	.
LSD[b]	4	3	6	4	9	9

[a] All herbicide treatments applied with a non-ionic surfactant at a 0.25% v/v ratio
[b] Abbreviations: Days after treatment, DAT; Fisher's least significant difference, LSD.
[c] NS, non-significant; *, **, ***, significant at the 0.05, 0.01 and 0.001 levels, respectivel

CONCLUSIONS

Results of this research are beneficial to both researchers and practitioners. Investigations into HPPD-inhibiting herbicides on bermudagrass benefit practitioners by identifying tembotrione and topramezone as herbicides most active against this problematic weed in cool-season turfgrasses. Researchers can use this information to further investigate tembotrione and topramezone as components of integrated bermudagrass control programs. These programs may involve mixtures of tembotrione and topramezone with other herbicides to provide commercially acceptable control and/or combining cultural practices such as interseeding to improve efficacy. These investigations also provide information to researchers on the relative value of visual evaluations and chlorophyll fluorescence yield measurement in determining activity of HPDD-inhibiting herbicides.

Other experiments demonstrated that nitrogen fertilizer enhances efficacy of mesotrione and topramezone against smooth crabgrass. These experiments provide turfgrass managers with specific rates of nitrogen and herbicides to provide acceptable crabgrass control. Results of this research will hopefully inspire other researchers to further investigate the physiological causes of this response and determine if it occurs in other weed species with different herbicides.

Field experiments investigating integrative approaches to control dallisgrass in tall fescue demonstrated that application timing affects herbicidal efficacy. Interseeding also improved control of dallisgrass suggesting programs using both chemical and cultural practices will be most successful. This will allow turfgrass managers to achieve acceptable dallisgrass control with fewer herbicide applications and hopefully inspire researchers to discover a physiological basis for seasonal variation in control.

VITA

Matthew Thomas Elmore was born on July 6, 1987 in Columbia S.C. to Donna and Jim Elmore. Raised in Lugoff, S.C., he eventually relocated with his family to Lincoln University, PA at the age of 5. He attended St. Mark's High School in nearby Wilmington, DE and graduated in 2005. Enrolling in the Turfgrass Science program at Penn State University, he graduated in 2009 with a B.S. degree. He began his M.S. degree in 2009 at the University of Tennessee. Matt plans to enroll as a Ph.D. student at the University of Tennessee after graduation.