Topramezone for bermudagrass control and mitigation of seashore paspalum and creeping bentgrass injury with additive products

by

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Abstract

Methods for managing bermudagrass (Cynodon spp.) invasion of seashore paspalum (*Paspalum vaginatum* Sw.) are limited. In 2016 and 2017, a field study at Auburn University evaluated 'Tifway' bermudagrass (Cynodon dactylon (L.) Pers. x C. transvaalensis Burtt Davy) control and 'Sea Spray' seashore paspalum injury following sequential applications of topramezone alone and in combination with triclopyr. Combining topramezone with triclopyr improves bermudagrass control and reduces seashore paspalum injury compared to topramezone alone. Topramezone + triclopyr at 14.6 + 690.4 g a.i. ha^{-1} applied sequentially controlled bermudagrass > 90% at 14 days after second application, however control decreased over time. Unfortunately, sequential applications of topramezone + triclopyr at 14.6 + 690.4 g a.i. ha^{-1} caused over 50% seashore paspalum injury and reduce turfgrass quality.

Creeping bentgrass (*Agrostis stolonifera* L.) response to topramezone is highly variable and is influenced by rates, tank mixtures, and environmental conditions. The specifics of such factors remain poorly understood. The objectives of this research were (1) to evaluate topramezone injury at different application timings; and (2) study the potential safening of topramezone via combination with paclobutrazol, Fe chelate or a turfgrass pigment. Results from these studies indicate early-season application of topramezone is safe to creeping bentgrass at all rates examined. Late-season topramezone application caused greatest creeping bentgrass injury. This is likely due to the high temperatures that stressed the turfgrass during this period. Application of topramezone in combination with paclobutrazol, Fe chelate, or turfgrass pigment reduced visual turfgrass injury compared to topramezone applied alone, with paclobutrazol and Fe chelate reducing creeping bentgrass injury the most. We conclude that early topramezone applications with combinations of paclobutrazol, Fe chelate, or turfgrass pigment could safely be used on creeping bentgrass putting greens.

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List of Abbreviations

ACCase	Acetyl-CoA carboxylase
DAA	Days after application
DAB	Days after the second application
DOXP	1-deoxy-D-xyulose 5-phosphate synthase
g a.i. ha^{-1}	Grams of active ingredient per hectare
HPPD	4-hydroxyphenylpyruvate dioxygenase
kg ha^{-1}	Kilograms per hectare
LHC	Light harvesting complexes
PDS	Phytoene desaturase
POST	Postemergence
PRE	Preemergence
RCB	Randomized complete block

Chapter 1. Introduction and Literature Review

Carotenoid-inhibiting herbicides

Carotenoids are lipid soluble pigments integrated into light harvesting complexes (LHC), along with chlorophyll, and perform the physiological roles of light-harvesting photoprotection. Light-harvesting photoprotection is the prevention of oxidative damage, or photoinhibition, to the LHC allowing plants to maintain efficient rates of photosynthesis (Hofmann et al. 1996; Croce et al. 1999; Sandmann 2001; McElroy et al. 2006). Carotenoids have a function as photoprotectants by sequestering free radicals before oxidative damage can occur to the chlorophyll and the larger LHC (Siefermann-Harms 1987; Armstrong and Hearst 1996; Niyogi 1999). They function as light-harvesting pigments by channeling photons unabsorbed by the chlorophyll molecule to the reaction center for photosynthesis (Croce et al. 1999; Demmig-Adams et al. 1996; Frank and Cogdell 1996). Two negative processes happen when carotenoids are inhibited – the LHC is destroyed due to light stress and light is not channeled to the chlorophyll for photosynthesis. This destroys the plant's ability to conduct photosynthesis.

The carotenoid biosynthesis pathway is an important target site for several classes of herbicides. Blockage of the terpenoid synthesis pathway at any point will result in inhibition of carotenoid synthesis, since carotenoids are formed later in the pathway (Duke 1990). As a primary effect, the herbicide seems to interfere with the desaturation steps of carotenoid biogenesis blocking the accumulation of colored carotenoids (Bartels and McCullogh 1972). Some herbicides block carotenoid biosynthesis by inhibiting phytoene desaturase (PDS), the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD), or 1-deoxy-D-xyulose 5-phosphate synthase (DOXP), a key component to plastid isoprenoid synthesis, in the case of clomazone (Lee et al. 1997; Mueller et al. 2000; Ferhatoglu and Barrett 2006).

Carotenoid biosynthesis inhibitors are also called bleaching herbicides because the inhibition of carotenoids causes bleaching of cells and plant foliage (Sandmann et al. 1990; Sandmann et al. 1991). However, thylakoids that have an intact pigment inventory before herbicide treatment are not affected by the application of bleaching herbicides. Typically, bleaching occurs only in newly formed leaves (Sandmann et al. 1991). The consequence of decreased carotenoid content, which normally protects chlorophyll from photooxidation under high light/low photosynthesis conditions, is chlorosis in new growth and the eventual death of the plant (Sandmann et al. 1990; Chamovitz et al. 1993).

Carotenoid biosynthesis inhibiting herbicides consist of both preemergence and postemergence herbicides applied on different weed manager systems as cropping, landscape, industrial use, and others. In general, they are active on broadleaf weeds but occasionally they will control select grasses.

HPPD-Inhibiting Herbicides

The enzyme 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27; HPPD) is a key component of photosynthesis because it catalyzes the transformation of 4-hydroxyphenylpyruvic acid (HPPA) into homogentisic acid (HGA) (Ndikuryayo et al. 2017). p-Hydroxyphenylpyruvate dioxygenase (HPPD) is the target site of β-triketone herbicides in current use (Dayan et al. 2009). Inhibition of HPPD causes photodynamic bleaching of the foliage as a result from an indirect inhibition of carotenoid synthesis due to the involvement of plastoquinone as a cofactor of phytoene desaturase (Pallett et al. 1998).

HPPD is the most recently developed herbicidal site of action (Mitchell et al. 2001; Grossman and Ehrhardt 2007; Duke 2012; Wang et al. 2015). Currently, there are about 13 HPPD-inhibiting herbicides commercially available, which are classified into three chemical families: triketones, pyrazoles, and isoxazoles (diketonitrile) (Ahrens et al. 2013; Wang et al. 2015).

The HPPD-inhibiting herbicides have many advantages, because they have PRE- and POST-emergence activity, excellent crop selectivity, low application rates, and low toxicity. They are also labeled for the control or suppression of a broad-spectrum of grass and broadleaf weeds, including weeds with resistance problems with other herbicides (Anonymous 2006; Smith et al. 2013; Wang et al. 2015).

HPPD enzymes is the target site of a number of important carotenoid biosynthesis inhibitor herbicides. Isoxaflutole, bicyclopyrone, mesotrione, tembotrione, topramezone pyrazolate, pyrazoxyfen, and pyrasulfatole are all HPPD inhibitors and are used for weed control in a variety of crop species (Luscombe and Pallett 1996; Mitchell et al. 2001; Matsumoto et al. 2002; Grossman and Ehrhardt 2007; Bollman et al. 2008; Sarangi and Jhala 2018; Hawkes et al. 2019), Several weeds controlled by HPPD inhibitors in crops are also found in turfgrass systems.

Mesotrione (Tenacity; Syngenta Crop Protection, LLC, Greensboro, NC) was the first HPPD-Inhibiting herbicide introduced to the turfgrass market. Mesotrione can be applied PRE- and/or POST-emergence at rates of 0.28 to 5.6 kg ha^{-1}. It is safe on Kentucky bluegrass (*Poa pratensis*), centipedegrass (*Eremochloa ophiuroides*), buffalograss (*Buchloe dactyloides*), fine fescue (*Festuca* spp.), tall fescue (*Festuca arundinacea*), perennial ryegrass (*Lolium perenne*), and St. Augustinegrass (*Stenotaphrum secundatum*) and it can be used to control more than 46 different broadleaf and grass weed species (Anonymous 2011).

Topramezone (Pylex^{MT} Herbicide 2.8C; BASF Corporation, Research Triangle Park, NC) is a new herbicide labeled for postemergence control of both grass and broadleaf weeds in most cool-season turfgrasses such as fescues (*Festuca* sp.), bluegrasses (*Poa* sp.), and ryegrasses (*Lolium* L.) on golf courses, sod farms and residential turfgrass (Anonymous 2006; Smith et al. 2013; BASF 2015; Haller et al. 2017). Topramezone has become an important tool in turfgrass systems as it effectively controls crabgrass species (*Digitaria* spp.) and goosegrass [*Eleusine indica* (L.) Gaertn.] at low application rates, from 12.3 to 36.8 g a.i. ha^{-1}.

Additionally, sequential applications of topramezone has potential to suppress bermudagrass one of the hardest weeds to control in turfgrass systems (Brosnan et al. 2011; BASF 2015). However, research has shown that topramezone alone cannot effectively control bermudagrass, because this species is very aggressive and has a high capacity for new infestation via regenerative underground organs (Beard 1973; Webster and Nichols 2012; McElroy and Breeden 2006). Reapplication and tank mixtures with other herbicides may be required to provide effective long-term control (Brosnan and Breeden 2013; McElroy and Breeden 2006).

Topramezone activity

In comparison to other HPPD-inhibiting herbicides, topramezone is a strong inhibitor of the 4-HPPD enzyme that controls a wide spectrum of annual grass and broadleaf weeds (Grossmann and Ehrhardt 2007). It’s a selective post-emergence herbicide that belongs to the pyrazolone chemical family that inhibits 4 hydroxyphenylpyruvate dioxygenase (4-HPPD) and the biosynthesis of plastoquinone, subsequently inhibiting carotenoid pigment formation, damaging membrane structure and disrupting chlorophyll (Grossmann and Ehrhardt 2007; Schönhammer et al. 2006). Soon after application, susceptible weeds bleach white because of chlorophyll loss and growth stops followed by necrosis and death (Apparao et at. 2015; Brosnan et at. 2011; Grossmann and Ehrhardt 2007).

Topramezone is rapidly absorbed by susceptible weeds via leaves, roots, and shoots and is translocated within the plants systemically through acropetal and basipetal movement (Grossmann and Ehrhardt 2007). Uptake by and translocation within the shoot is significantly increased with a suitable adjuvant (Schönhammer et al. 2006). Significant increase of foliar absorption and translocation of topramezone by plants was observed when topramazone was mixed with methylated seed oil (MSO) opposed to being applied alone (Grossmann and Ehrhardt 2007; Zhang et al. 2013). Xu et al. (2011) reported that MSO decreases the surface tension and contact angle resulting in increased surface coverage area on both waxy and hairy leaves. However, information about the mechanism of adjuvant enhancing the biological efficacy of topramezone on weeds remains poorly understood.

Topramezone's moderate water solubility and moderate persistence in soil allows root uptake by plants (Gorsic et al. 2008; Rahman et al. 2014). The influence of environmental factors affecting sensitivity of plants to topramezone has been reported (Grossmann and Ehrhardt 2007; Williams and Pataky 2010). Topramezone applications during favorable growing conditions are recommended to optimize turfgrass tolerance and weed control. Turfgrass under environmental stress is more likely to show injury. Turfgrass injury to topramezone can be influenced by different factors such as application rate, tank mixtures, growing season, soil/air temperature, precipitation, turfgrass species and other environment conditions (Brosnan et al. 2013; Elmore et al. 2015a; Brewer et al. 2016; Johnston et al. 2016; Cox et al. 2017). The specifics of such factors remain poorly understood.

Safe applications

Herbicides are the cheapest and fastest method of weed control in agricultural systems. Satisfactory control occurs when herbicides are used at their correct application rate for control of target weeds. The current trend is to use products at the lowest effective application rate that is safe for non-target species and the environment (Arya 2005; Lee et al. 2015; Marble et al. 2015; Powles and Yu 2010). However, selective weed control in turfgrass systems presents unique challenges not present in other cropping systems. Turfgrass managers need to select and apply herbicides that provide adequate weed control with minimum turfgrass phytotoxicity because aesthetic quality, not yield, is the primary focus for turfgrass managers (Marble et al. 2015; Wolfe et al. 2016; Yu et al. 2019a, 2019b).

Herbicide safeners (also known as antidotes) have been used to protect crops from herbicide phytotoxicity without reducing activity in target weed species, increasing the margin of selectivity, and expanding the window of application for several herbicides (Davies and Caseley 1999; Davies 2001; Hatzios and Burgos 2004; Elmore et al. 2015b; Elmore et al. 2016). Herbicide safener selectivity is dependent on either application placement or differential herbicide safener interactions among crops and weeds. Safeners are most effective when applied either before or simultaneously with the herbicide (Hatzios and Burgos 2004). Some herbicides are used as safeners of other herbicides, promoting the reduction of crop injury and extending the time of application (Moran et al. 2011).

Recently, topramezone efficacy and safening applications have been the focus of topramezone research in turfgrass. Tank-mixing topramezone with triclopyr reduces bleaching injury without compromising efficacy (Brosnan and Breeden 2013; Brosnan et al. 2013; Cox et al. 2017). Elmore et al. (2015a and 2015b) found that the herbicide safener cloquintocet-mexyl (cloquintocet) applied in combination with topramezone reduces topramezone-induced creeping bentgrass (*Agrostis stolonifera* L.) injury and also increased creeping bentgrass biomass and PSII quantum yield. The researchers suggested that creeping bentgrass tolerance to topramezone is influenced by cytochrome P450-catalyzed metabolism (Elmore et al. 2015b). Researchers at Auburn University, Virginia Tech, BASF, and other universities have suggested that topramezone can be commercially viable in moderately tolerant turfgrass species such as seashore paspalum (*Paspalum vaginatum* Sw.) and creeping bentgrass (*A. stolonifera* L.) by using tank mixtures with safening compounds.

Research Objectives

1. Evaluate bermudagrass control and seashore paspalum injury to topramezone and triclopyr alone and in combination.

2. Determine environmental influences and optimal growing season to topramezone application for creeping bentgrass (*Agrostis stolonifera* L.).

3. Evaluate the integration of field topramezone applications with paclobutrazol (Trimmit 2SC), Fe chelate (Sprint 330) or a turfgrass pigment (Sarge 2.0) to prevent undesirable creeping bentgrass (*Agrostis stolonifera* L.) bleaching injuries.

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Chapter 2.

Response of seashore paspalum (*Paspalum vaginatum* Sw.) and hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis* L. Pers.) to topramezone and triclopyr mixtures

The following chapter was formatted to facilitate publication in *Weed Technology*.

Chapter 2. Response of seashore paspalum (*Paspalum vaginatum* Sw.) and hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis* L. Pers.) to topramezone and triclopyr mixtures

Abstract

There are few options available for controlling bermudagrass (Cynodon spp.) invasion of seashore paspalum (*Paspalum vaginatum* Sw.). The control of 'Tifway' bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt Davy) and tolerance of seashore paspalum to topramezone, triclopyr, or the combination of these two herbicides were evaluated in both greenhouse and field condition. Field treatments included two sequential applications of topramezone (15.6 g a.i. ha^{-1}) alone and five rates of topramezone + triclopyr (15.6 + 43.2, 15.6 + 86.3, 15.6 + 172.6, 15.6 + 345.2, or 15.6 + 690.4 g a.i. ha^{-1}). Secondary greenhouse treatments included a single application of topramezone (20.8 g a.i. ha^{-1}) or triclopyr (258.9 g a.i. ha^{-1}) alone, or in combination at 20.8 + 258.9 or 20.8 + 517.8 g a.i. ha^{-1}, respectively. Field and greenhouse results showed that topramezone applications in combination with triclopyr improves bermudagrass control and reduces seashore paspalum bleaching injury compared to topramezone alone. In field evaluations topramezone + triclopyr at 15.6 + 690.4 g a.i. ha^{-1} in sequential applications controlled bermudagrass > 90%, however, control decreased over time. Further, sequential applications of topramezone + triclopyr at 15.6 + 690.4 g a.i. ha^{-1} cause > 50% seashore paspalum injury. Topramezone plus triclopyr application programs have potential for increasing bermudagrass control and reducing seashore paspalum injury, however, such

programs will likely require manipulation of the rate of topramezone, application timing initiation, application interval, and number of applications, in order to maximize control and minimize injury.

Nomenclature: *Cynodon dactylon* x. *C. Transvaalensis* L. Pers.; *Paspalum vaginatum* Sw.; HPPD (enzyme *p*-hydroxyphenylpyruvate dioxygenase); Topramezone ([3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone); Triclopyr (3,5,6-trichlor o-2-pyridinyl-oxyacetic acid).

Key words: Efficacy, HPPD, injury, tank-mixture, synergist, Turfgrass, weed control.

Introduction

Seashore paspalum is a warm-season turfgrass primarily utilized in coastal southern U.S. and similar regions around the world (Duncan and Carrow 2002; Raymer et al. 2008). In addition to desirable qualities such as dark-green color, fine texture, tolerance to low-mowing, and traffic tolerance (Brosnan and Deputy 2009; Trenholm et al. 1999, 2000); seashore paspalum is also well adapted to environmental stresses, including high salt concentrations, drought, flooding, wide pH ranges (4.0 to 10.2), and low light (Duncan 1999; Jiang et al. 2004; McCullough and Raymer 2011; Shahba et al. 2012).

Bermudagrass often invades seashore paspalum, reducing overall turf quality and decreasing the long-term sustainability of seashore paspalum. With its aggressive stolons and rhizomes, bermudagrass is one of the most difficult weeds to control in turfgrass systems (Beard 1973; McElroy and Breeden 2006; Webster and Nichols 2012). Selective control often requires multiple applications over multiple years. To selectively control bermudagrass in turfgrass, herbicides such as ethofumesate plus flurprimidol, topramezone, mesotrione, fenoxaprop, fluazifop alone or in combination with triclopyr have been used (Brosnan and Breeden 2013; Johnson and Duncan 2000; McElroy and Breeden 2006). However, unacceptable turfgrass injury can occur with these herbicides depending on application timing, frequency, and rate.

Labeled and unlabeled herbicides safe to apply to seashore paspalum include: carfentrazone, dithiopyr, halosulfuron, oxadiazon, prodiamine, clopyralid, metsulfuron, quinclorac, pronamide, bentazon, dicamba, imazaquin, and mecoprop + 2,4-D + dicamba (Unruh et al. 2006; Patton et al. 2009; McCullough et al. 2012). However, none of these herbicides will control bermudagrass in seashore paspalum. Ethofumesate is the only potential herbicide for bermudagrass control in seashore paspalum, however, seashore paspalum injury is excessive for most situations (Johnson and Duncan 2000; Johnson and Duncan 2003; Unruh et al. 2006; McCullough et al. 2016).

Topramezone ([3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone) is a new carotenoid biosynthesis inhibitor that specifically, inhibits *p*-hydroxyphenylpyruvate dioxygenase (HPPD). Topramezone is utilized in corn as Impact® and Armezon® and turfgrass as Pylex^MT for the control of both grass and broadleaf weeds (Anonymous 2006; Grossmann et al. 2007; Bollman et al. 2008). Among HPPD herbicides mesotrione, topramezone, and tembotrione, topramezone and tembotrione have potential for bermudagrass suppression (Brosnan et al. 2011), though topramezone is considered most active (Elmore et al. 2011a). Previous research indicates no HPPD inhibitor herbicide alone effectively controls bermudagrass, suggesting tank mixtures with other modes of action may be required to provide effective long-term control of bermudagrass.

Triclopyr (3,5,6-trichlor o-2-pyridinyl-oxyacetic acid) is a synthetic auxin herbicide that controls bermudagrass. Several studies indicate control potential increases when used in combination with other herbicides (McElroy and Breeden 2006; Brosnan and Breeden 2013; Lewis et al. 2010; Doroh et al. 2011). For instance, for bermudagrass invasion in zoysiagrass, triclopyr in combination with fluazifop or fenoxaprop increases bermudagrass control and also reduces zoysiagrass injury; this synergism both improves control of the target and reduces injury to the non-target turfgrass (McElroy and Breeden 2006). Triclopyr significantly increases bermudagrass control when applied in combination with topramezone compared to topramezone or triclopyr alone (Brosnan and Breeden 2013).

Previous research shows topramezone combined with triclopyr provides bermudagrass control in zoysiagrass and tall fescue (Brosnan and Breeden 2013; McElroy and Breeden 2006).

However, no study has been conducted on their efficacy in seashore paspalum. Our research objective was to evaluate bermudagrass control and seashore paspalum injury to topramezone, triclopyr alone and these herbicides in combination.

Materials and Methods

Research was conducted at Auburn University Sports Surface Field Laboratory and Weed Science greenhouse in Auburn, Alabama to evaluated bermudagrass control and seashore paspalum tolerance to topramezone (Pylex^{MT} Herbicide 2.8C; BASF Corporation, Research Triangle Park, NC) and triclopyr (Turflon Ester 4SL; Dow AgroSciences LLC). Both field and greenhouse experiments were conducted to achieve this goal. The turfgrass varieties were Tifway bermudagrass (*Cynodon dactylon* x. *C. Transvaalensis* L. Pers.) and Sea Spray seashore paspalum (*Paspalum vaginatum* Sw.).

Field Trials. Field research was conducted at the Sports Surface Field Laboratory in Auburn, AL between September to November of 2016 and repeated from August to October of 2017. The soil type was a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) with a pH of 6.1 and 1.5% organic matter (OM), fertilizer applied monthly at 24.4 kg ha^{-1} N in liquid urea form and irrigation applied as needed to promote turfgrass health.

Treatments were a combination of topramezone and triclopyr (Table 1) applied twice on an 18-day interval. Herbicide treatments were applied with a CO_2 pressurized sprayer calibrated to deliver 280 L ha^{-1} with a handheld four-nozzle boom (TeeJet TP8003VS nozzles with 25 cm spacing; Spraying Systems Company, Wheaton, IL) to 1.52 by 1.52 m plots. A nonionic surfactant was included with all treatment at 0.25% v/v. Plots were not mowed the day before or after application.

Visual ratings were made 3, 7, and 14 days after the first herbicide application and 3, 7, 14, 28, and 42 days after the second herbicide application. Both bermudagrass control and seashore paspalum injury were evaluated relative to the non-treated on a 0 (no injury) to 100 (complete plant death) scale. Seashore paspalum injury greater than 20% was considered commercially unacceptable.

Experimental design was randomized complete block (RCB) replicated four times. Data were subjected to ANOVA using SAS 9.4 (SAS Institute Inc., Cary, NC). Year by treatment interactions ($P>0.05$) were detected for bermudagrass control and seashore paspalum injury; therefore, the years are presented separately. While this statistical difference was observed, our assessment of the data was that years are visually similar and extrapolation with both years is warranted. The result of each replicate was expressed as mean and standard error of mean (±SEM) of four independent replicates. Graphs were plotted with Sigma Plot 10.0 program (Systat Software Inc).

Greenhouse Trials. Greenhouse trials were conducted in conditions of 32/27 C (+/-3 C day/night) with average relative humidity 68%. The trials were conducted from February to May 2019. Single plug (5 cm diameter) of bermudagrass and seashore paspalum were transplanted individually into plastic pots (10 cm diameter) filled with a Marvyn sandy loam soil (fine-loamy, kaolinitic, thermic Typic Kanhapludults) with a pH of 6.5 and 1.1% organic matter. Pots were irrigated three times a day with overhead irrigation and fertilized every two weeks to promote growth.

Treatments were topramezone and triclopyr application alone and tank mix (Table 1). Nonionic surfactant (Induce, Helena® Chemical Company, Collierville, TN) at 0.25% v/v was included in all treatments. Treatments applications were made with a CO_2 pressurized sprayer calibrated to deliver 280 L ha^{-1} with a handheld four-nozzle boom (TeeJet TP8003VS nozzles with 25 cm spacing; Spraying Systems Company, Wheaton, IL). Bermudagrass control and seashore paspalum injury, photochemical efficiency (F_v/F_m), and clipping weight were measured once a week at 7, 14, 21, 28, 35 ,42, and 48 DAT. Both bermudagrass control and seashore paspalum injury were evaluated relative to the non-treated with similar scale of field trials. Clipping weight was measured once a week for determinations treatments impact on bermudagrass and seashore paspalum growth. F_v/F_m were assessed with a chlorophyll fluorometer designed for light adapted yield (OS1-FL Portable | Opti-Sciences, Inc., Hudson, NH). The measurements were made approximately 0.5 cm from the bermudagrass and seashore paspalum leaves between 11:30 AM - 12:30 PM (central time zone). at midday (between 11:30 A.M. - 12:30 P.M. Central Time Zone).

The experimental design was completely randomized factorial design replicated four times and was repeated twice. Effects due to herbicide, application rate, and interactions were subjected to ANOVA at the 0.05 probability level using SAS 9.4 (SAS Institute Inc., Cary, NC). The F_v/F_m data were presented in percent relative to the nontreated. Negative % F_v/F_m indicates a decrease in photochemical efficiency. Injury, F_v/F_m, and clipping weight were expressed as mean and standard deviation of mean (±SEM) of eight independent replicates. Figures were plotted with Sigma Plot 10.0 program (Systat Software Inc).

Results and Discussion

Field Trials. In general, topramezone alone induced bermudagrass bleaching with minimal necrosis, which resulted in unacceptable control by study conclusion (Figure 1a and 1b). In 2016, topramezone alone controlled bermudagrass approximately 68% at 14 days after the first application (DAA) (Figure 1a). In 2017, topramezone alone controlled bermudagrass approximately 85% at 7 DAA, but decreased to less than 40% at 14 DAA (Figure 1b).

Sequential applications of topramezone alone increased initial bleaching but did not improve bermudagrass control 42 days after the second herbicide application (defined as DAB) (Figure 1a and 1b). In 2016, bermudagrass control increased after the sequential application, reaching 76% at 14 DAB. Similarly, in 2017, bermudagrass control was 85% at 7 DAB. In both years bermudagrass recovered from topramezone alone injury. In both 2016 and 2017, control was < 50% at 28 DAB with continued decreasing injury until 42 DAB. Brosnan et al. (2011) also indicated topramezone at 0.018, 0.025 and 0.038 kg a.i. ha^{-1} provided 50% bermudagrass control, but visual bleaching decreased for all rates after 21 days. Other researchers report similar symptoms following application of the HPPD inhibitor herbicide mesotrione on other turfgrasses (Elmore et al. 2011a; McCurdy et al. 2008; Willis et al. 2007).

Topramezone in combination with triclopyr increased bermudagrass control at all rates, although none of the combined treatments completely controlled bermudagrass with only one application. In 2016, topramezone + triclopyr controlled bermudagrass approximately 86 and 94% at 15.6 + 345.2 and 15.6 + 690.4 g a.i. ha^{-1}, respectively, at 14 DAA (Figure 1a). Topramezone + triclopyr combinations at 15.6 + 43.2, 15.6 + 86.3, and 15.6 + 172.6 g a.i. ha^{-1}, controlled bermudagrass 73%, 71%, and 79%, respectively, at 14 DAA (Figure 1a). In 2017, up to 14 DAA, there was also a significant increase in bermudagrass control at all combined rates of topramezone + triclopyr. However, only topramezone + triclopyr at 15.6 + 690.4 g a.i. ha^{-1} controlled bermudagrass > 90% at 14 DAA in 2017 (Figure 1b). All other combinations, controlled bermudagrass less than 80% 14 DAA.

Sequential application of topramezone + triclopyr increased bermudagrass control in both years. In 2016, all combination rates controlled bermudagrass > 80% at 14 DAB (Figure 1a). Topramezone at 15.6 g a.i. ha^{-1} + triclopyr at 172.6, 345.2, or 690.4 g a.i. ha^{-1} controlled bermudagrass ≥ 95%, while, in 2017, only rates of 15.6 + 345.2 and 15.6 + 690.4 g a.i. ha^{-1} controlled ≥ 90% (Figure 1b). In both years, bermudagrass control decreased after 28 DAB for all topramezone + triclopyr treatments.

In agreement with Brosnan and Breeden (2013), topramezone combined with triclopyr significantly increased bermudagrass control compared to topramezone or triclopyr alone. Results of the present study indicate combining topramezone + triclopyr reduces also visual bleaching caused by topramezone alone and induces more necrotic symptoms to bermudagrass. Similarly, Brosnan et al. (2013) reported that triclopyr with topramezone reduces leaf bleaching in smooth crabgrass without reducing control. Decreased bleaching is important because this type of injury caused by HPPD inhibitor herbicides may be deemed unacceptable by turfgrass managers or the general public.

Several studies have indicated the synergism of carotenoid-inhibiting herbicides and triclopyr. The addition of triclopyr increased mesotrione efficacy for controlling multi-tiller smooth crabgrass in tall fescue, and addition of triclopyr reduced bleaching of the smooth crabgrass (Yu and McCullough 2016). Triclopyr also provides a synergistic effect when combined with herbicides that inhibit the acetyl-CoA carboxylase (ACCase) enzyme (McElroy and Breeden 2006; Lewis et al. 2010; Doroh et al. 2011).

Topramezone alone caused unacceptable injury to seashore paspalum. In both years, topramezone alone injured seashore paspalum more than 65% at 14 DAA and more than 70% at 14 DAB (Figure 2a and 2b). Similar to bermudagrass, injury was primarily bleaching of leaf tissue. By 42 DAB, seashore paspalum recovered from topramezone alone injury to levels deemed acceptable which were at or below all other treatments (Figure 2a and 2b). Triclopyr reduced bleaching caused by topramezone alone at all rates tested. In 2016, all topramezone + triclopyr treatments resulted in less than 25% injury on seashore paspalum until after sequential application. Likewise, in 2017 seashore paspalum injury was less than 30% until after the sequential application (Figure 2b).

Sequential application of topramezone + triclopyr increased injury from a single application (Figure 2). In both years, highest seashore paspalum injury followed lowest triclopyr rate (43.2 g a.i. ha^{-1}) and the highest rate (690.4 g a.i. ha^{-1}) across the length of the study. Topramezone at 15.6 g a.i. ha^{-1} + triclopyr at 86.3, 172.6, or 345.2 g a.i. ha^{-1} resulted in < 50% seashore paspalum injury. Injury decreased for all treatments after 28 DAB. In 2017, seashore paspalum recovered faster from herbicides injury (Figure 2b).

Greenhouse Trials. Treatment by experimental run interactions was nonsignificant ($P > 0.05$) for control and injury, F_v/F_m, and clipping weight; thus, data are pooled over experimental runs. Topramezone 20.8 g a.i. ha^{-1} or triclopyr 258.9 g a.i. ha^{-1} alone did not control bermudagrass (Figure 3a). Control was < 70% from single application throughout of the trial conduction. Single application of topramezone tank-mixture with triclopyr (20.8 + 258.9 or 20.8 + 517.8 g a.i. ha^{-1}) controlled total bermudagrass shoots tissue 21 DAA (Figure 3a). However, we observed after 35 DAA bermudagrass started regrowth by the regenerative underground organs, so reapplication would be required for long-term control (Appendix A).

The F_v/F_m values for nontreated bermudagrass ranged from 0.658 to 0.713 throughout the trials. Topramezone alone reduced bermudagrass F_v/F_m greater than triclopyr alone. Topramezone reduced F_v/F_m > 60% at 28 DAA, however triclopyr reduced F_v/F_m < 20% at the same time (Figure 3b). Topramezone alone reduced F_v/F_m more than triclopyr alone because of the site of action of topramezone results in primary inhibition of carotenoid biosynthesis coupled to secondary inhibition of chlorophyll biosynthesis and directly reduced the photosynthesis plants efficiency (Hess 2000; Brosnan et al. 2011). However, triclopyr, a synthetic auxin herbicide is based on their growth-promoting effects observed in plant cell, showed no direct effect on photosynthesis activities (Matsue et al.1993; Sterling and Hal 1997).

Topramezone in combination with triclopyr reduced F_v/F_m 100% at 21 DAA due to the total death of photosynthetic tissues. Although after 35 DAA a new regrowth was observed, there was not enough to measure F_v/F_m.

Compared with the nontreated, topramezone or triclopyr result in bermudagrass clipping weight reduction at 7, 14, and 21 DAA, but control is limited, and bermudagrass recovered after 28 DAA (Figure 3c). Total bermudagrass growth suppression was only achieved with topramezone with triclopyr tank-mix (Appendix A), but bermudagrass control decreased as recover stage increased at 48 DAA. Although topramezone and triclopyr tank-mix controlled acceptable bermudagrass, it is recommended sequential applications over multiple years as bermudagrass will continue to recover from rhizomes (Lewis et al. 2010; Brosnan and Breeden 2013).

Topramezone or triclopyr alone or tank-mix combination caused seashore paspalum injury more than 40% under greenhouse conditions (Figure 4a). Topramezone 20.8 g a.i. ha^{-1} alone or in combination with triclopyr (20.8 + 517.8 g a.i. ha^{-1}). injured seashore paspalum > 60% at 28 DAA. The lowest injury was observed for topramezone + triclopyr (20.8 + 258.9 g a.i. ha^{-1}). This suggests that lower application rates would be safe for use to avoid seashore paspalum injury (Appendix A).

The F_v/F_m values for nontreated seashore paspalum ranged from 0.694 to 0.739 throughout the trials. Topramezone treatment reduced seashore paspalum F_v/F_m greater than triclopyr at each rating date. Topramezone reduced $F_v/F_m \geq 40\%$ at 21, 28, and 35 DAA (Figure 4b) and triclopyr reduced $F_v/F_m < 15\%$ at all rating date. Topramezone with triclopyr tank-mix increase F_v/F_m compared to topramezone applied alone at 21, 28, and 35 DAA.

Our results indicated that the increase in bleaching injury caused by topramezone coincided with reductions in F_v/F_m. Similar reductions in F_v/F_m have been observed of bermudagrass leaf tissue treated with mesotrione, topramezone, and tembotrione (Elmore et al. 2011b). Based on scientific literature, F_v/F_m is quantification of the photosystem II efficiency, and photosystem II efficiency reduction can often be the first manifestation of stress on the leaf. (Maxwell and Johnson 2000; McElroy and Walker 2009).

Topramezone alone reduced seashore paspalum clipping weight less than triclopyr alone or tank-mix (Figure 4c). This result was expected because plant growth regulator herbicides such triclopyr have been used on roadsides rights-of-way to reduce mowing requirements (Jeffries et al. 2017).

Research Implication. It is apparent from this study, hybrid bermudagrass cannot be controlled with two applications of topramezone with or without triclopyr. What is demonstrated, however, is the possibility of using topramezone with or without triclopyr as a component in a program to control bermudagrass in seashore paspalum. There are currently no single application programs for bermudagrass control in any scenario. Even glyphosate requires sequential or multiple applications to provide long-term acceptable bermudagrass control (Johnson 1988).

Selective bermudagrass control in other turfgrass species is largely achieved with multiple applications over multiple years (Johnson and Carrow 1995; McCarty 1996). Such scenarios allow bermudagrass removal from a turfgrass stand and the simultaneous growth into the killed areas by the desirable turfgrass. Acceptable bermudagrass control has been achieved followed sequential application of ethofumesate plus atrazine applied in March, April and May, which had no effect on the St. Augustungrass quality (McCarty, 1996). Fenoxaprop plus ethofumesate (0.2 + 1.7 kg ha^{-1}) can be used in tall fescue to bermudagrass control, although injury occurs, the symptoms will appear for 1 to 2 wk following treatment but tall fescue recovers completely. (Johnson and Carrow 1995). Clodinafop, fenoxaprop, and metamifop tank-mixed with triclopyr can be applied in zoysiagrass and which controls bermudagrass greatly than 89% (Doroh et al. 2011)

Topramezone could potentially be integrated into such bermudagrass control programs. In our estimation, such programs will likely require manipulation of the rate of topramezone, application timing initiation, application interval, and number of applications. Further, we have demonstrated that triclopyr is a possible synergist for bermudagrass control that also reduces seashore paspalum injury, at least in the short term.

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Table 1. Active ingredient and trade name, along with active ingredient rates utilized in the studies.

Treatment	Herbicide active ingredient	Trade name	Rate g a.i. ha^{-1}
		Field Trials	
1	Non-treated	-	0.0
2	Topramezone	Pylex	15.6
3	Topramezone + Triclopyr	Pylex + Turflon Ester	15.6 + 43.2
4	Topramezone + Triclopyr	Pylex + Turflon Ester	15.6 + 86.3
5	Topramezone + Triclopyr	Pylex + Turflon Ester	15.6 + 172.6
6	Topramezone + Triclopyr	Pylex + Turflon Ester	15.6 + 345.2
7	Topramezone + Triclopyr	Pylex + Turflon Ester	15.6 + 690.4
		Greenhouse Trials	
1	Non-treated	-	0.0
2	Topramezone	Pylex	20.8
3	Triclopyr	Turflon Ester	258.9
4	Topramezone + Triclopyr	Pylex + Turflon Ester	20.8 + 258.9
5	Topramezone + Triclopyr	Pylex + Turflon Ester	20.8 + 517.8

Nonionic at 0.25% v/v was included in all treatments.

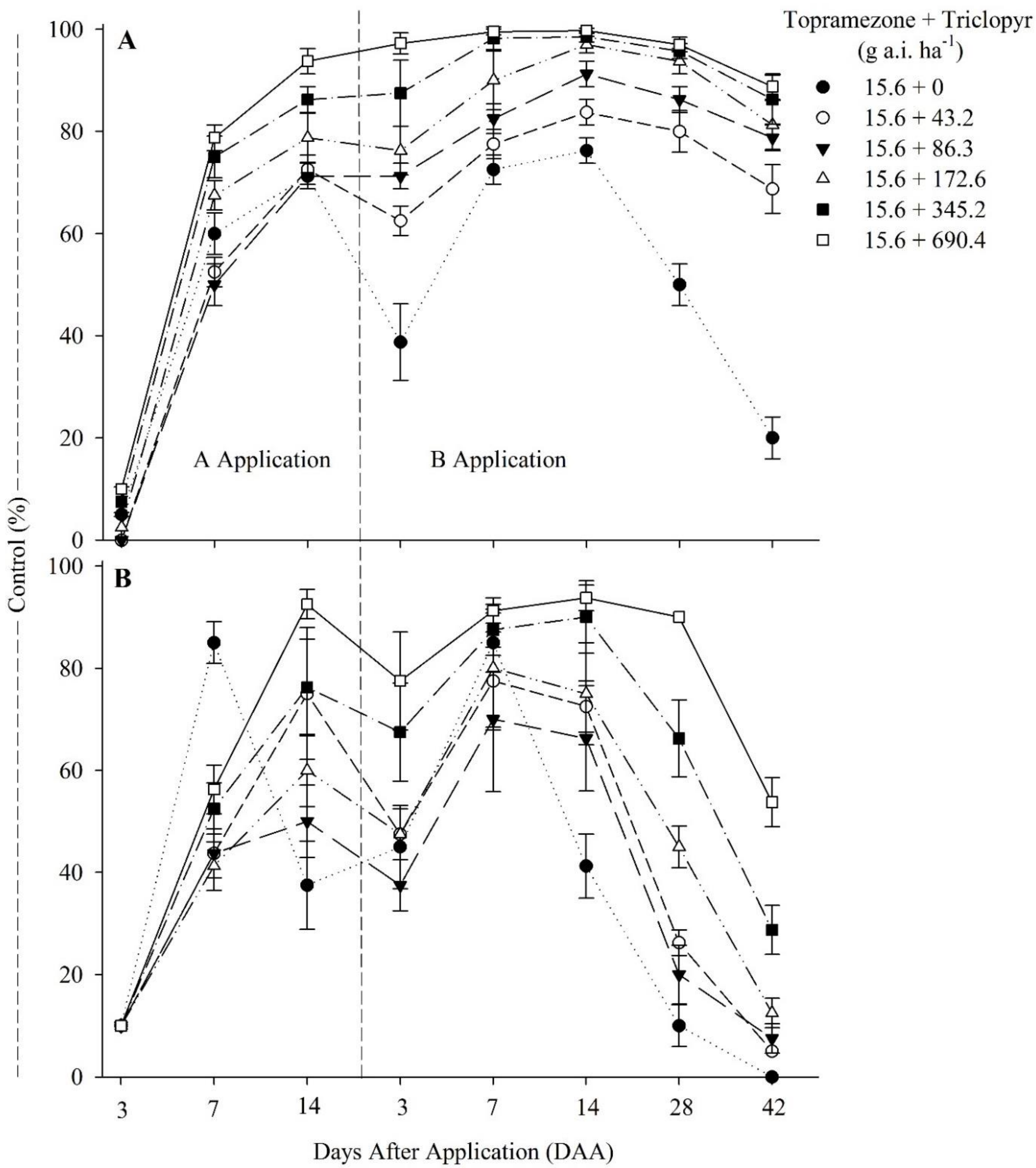

Figure 1. Control of bermudagrass treated with topramezone + triclopyr at 3, 7 and 14 days after application (DAA) and 3, 7, 14, 21 28 and 42 days after second application (DAB). Research from 2016 (A), research from 2017 (B). Error bars indicate standard errors of individual means, *n = 4.*

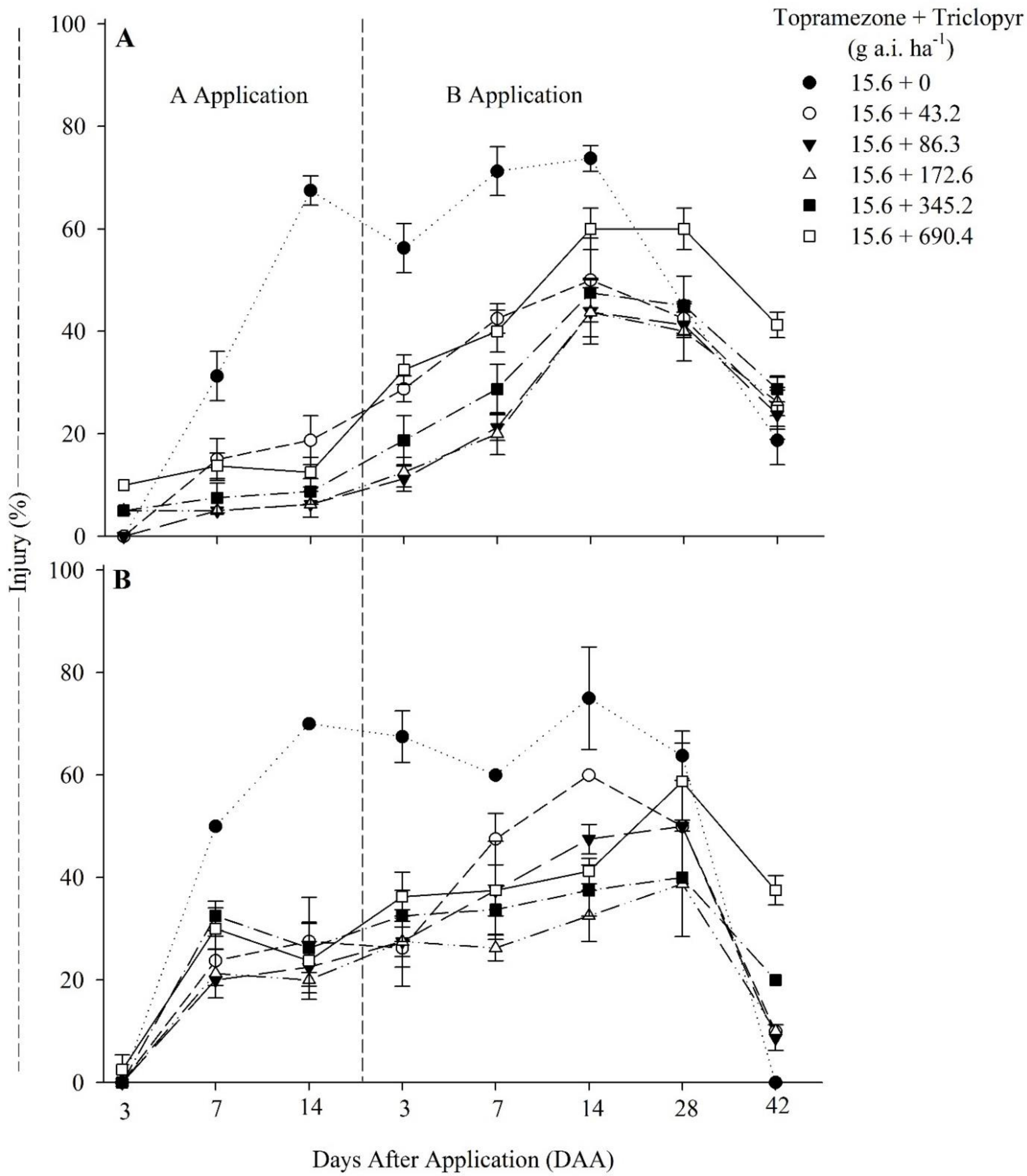

Figure 2. Injury of seashore paspalum treated with topramezone + triclopyr at 3, 7, and 14 DAA and 3, 7, 14, 28, and 42 DAB. Research from 2016 (A), research from 2017 (B). Error bars indicate standard errors of individual means, *n = 4.*

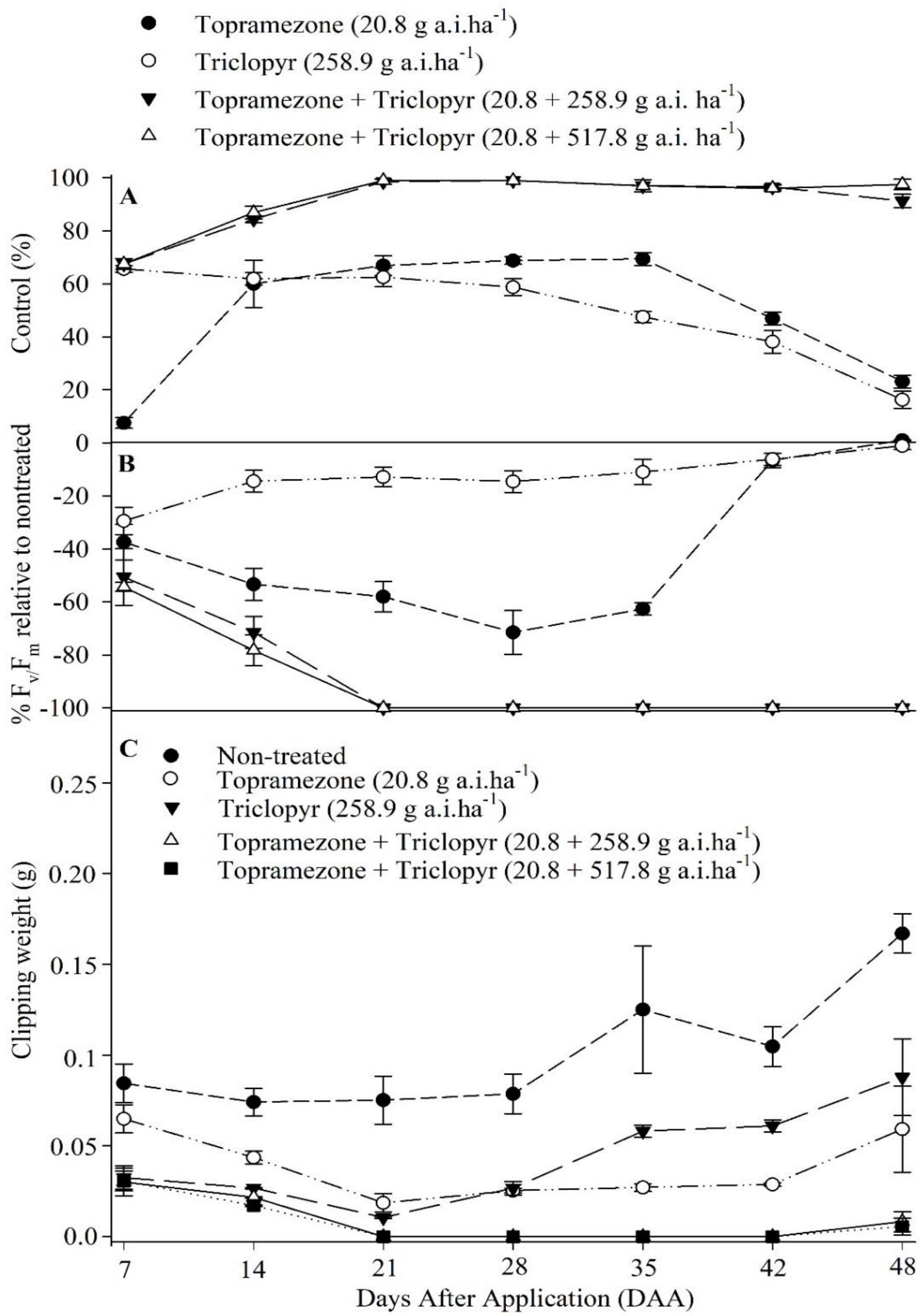

Figure 3. Control (%) (A), chlorophyll fluorescence (F_v/F_m) (B), and clipping weight (g) (C) of bermudagrass treated with topramezone or triclopyr alone or in combination at 7, 14, 21, 28, 35, 42, and 48 days after application (DAA). Error bars indicate standard errors of individual means, *n = 8.*

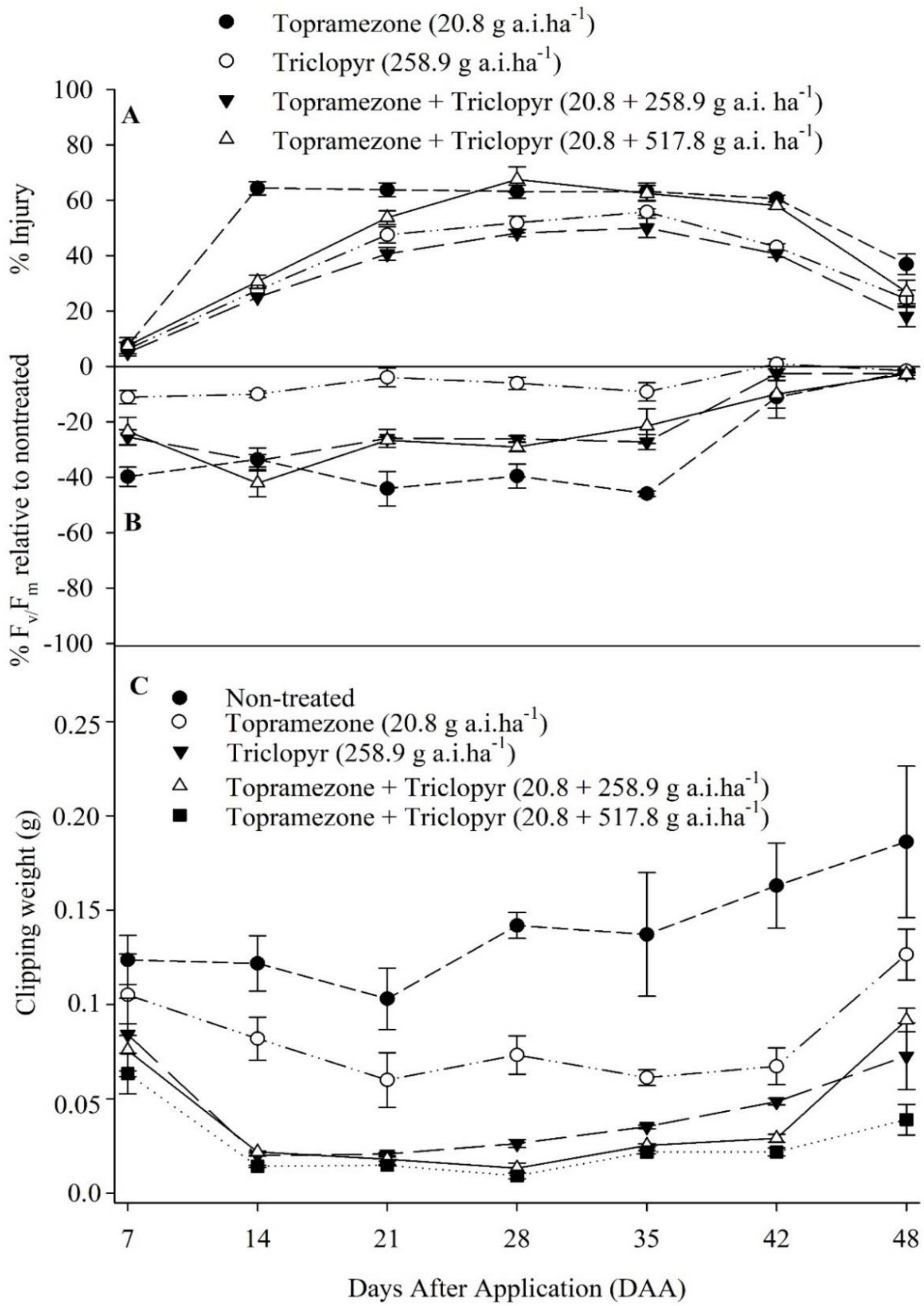

Figure 4. Injury (%) (A), chlorophyll fluorescence (F_v/F_m) (B), and clipping weight (g) (C) of seashore paspalum treated with topramezone or triclopyr alone or in combination at 7, 14, 21, 28, 35, 42, and 48 days after application (DAA). Error bars indicate standard errors of individual means, *n = 8.*

Chapter 3.

Influence of timing and safening application of topramezone on creeping bentgrass (*Agrostis stolonifera* L.) with additive product combinations

The following chapter was formatted to facilitate publication in *Weed Technology*.

Chapter 3. Influence of timing and safening application of topramezone on creeping bentgrass (*Agrostis stolonifera* L.) with additive product combinations

Abstract

Creeping bentgrass response to topramezone is highly variable and is influenced by rates, tank mixtures, and environmental conditions. The specifics of such factors remain poorly understood. The objectives of this research were to evaluate (study 1) topramezone injury at different application timings and (study 2) potential safening of topramezone via combination with paclobutrazol, Fe chelate or a turfgrass pigment. Field treatments for experiment 1 included applications of topramezone alone at 5.2, 10.4, 15.6 or 20.8 g a.i. ha^{-1}) in April, May, June, or July. For experiment 2, three sequential applications of topramazone (5.2 or 10.4 g a.i. ha^{-1}) alone and in combination with paclobutrazol (98.1 g a.i. ha^{-1}), Fe chelate (610.3 g a.i. ha^{-1}), or turfgrass pigment (1.68 kg ha^{-1}) were made. Greenhouse experiments included topramezone applied alone, in tank mixture, three days before or three days after: paclobutrazol, Fe chelate or turfgrass pigment applications. Topramezone applied at four different rates alone or in a tank mixture with paclobutrazol was also evaluated in greenhouse studies. Results from these studies indicate early-season application of topramezone is safe to creeping bentgrass at all rates examined. Late-season topramezone application caused greatest creeping bentgrass injury. This is likely due to the high temperatures that stressed the turfgrass during this period. In general, injury from topramezone increased from April to July applications. Application of topramezone in combination with paclobutrazol, Fe chelate, or turfgrass pigment reduced visual turfgrass injury compared to topramezone applied alone. Treatments that included paclobutrazol and Fe chelate application programs were the most effective because they increased turfgrass quality and reduced bleaching injury caused by topramezone. We conclude that early topramezone applications with combinations of paclobutrazol, Fe chelate, or turfgrass pigment could safely be used on creeping bentgrass putting greens.

Nomenclature: *Agrostis stolonifera* L.; HPPD (enzyme *p*-hydroxyphenylpyruvate dioxygenase); Topramezone ([3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone).

Key words: Efficacy, HPPD, injury, safener, tank-mixture, turfgrass.

Introduction

Weed infestation is a serious issue in all cropping systems. However, selective weed control in turfgrass presents unique challenges not present in other cropping systems. Turfgrass managers must select herbicides that provide adequate weed control with minimum turfgrass phytotoxicity because herbicides used in turfgrass systems are applied primarily for maintaining aesthetic quality and not solely to reduce weed competition or improve yields (Marble et al. 2015; Wolfe et al. 2016; Yu et al. 2019a, 2019b).

Carotenoid inhibitors have developed into niche herbicides within turfgrass systems due to the need for repeat applications and potential for bleaching green tissue white, even on tolerant species (Hess 2000; McElroy and Walker 2009a; Brewer et al. 2016). Despite these issues, carotenoid inhibitors remain a viable weed control option, especially in cool-season grasses (McElroy et al. 2007; McCurdy et al. 2008; Brosnan et al. 2013; Yu and McCullough 2016).

Topramezone ([3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone) is a new carotenoid biosynthesis inhibitor in the pyrazole chemical family. Specifically, topramezone (Pylex^{MT}) inhibits the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD) (Anonymous 2006; Grossmann et al. 2007). Topramezone is a postemergence herbicide labeled for safety in most cool-season turfgrasses such as fescues (*Festuca* sp.), bluegrasses (*Poa* sp.), and ryegrasses (*Lolium* L.) (BASF 2015; Haller et al. 2017)

Recently, topramezone efficacy and safety has been the focus of topramezone research in turfgrass. Tank-mixing topramezone with triclopyr, reduces bleaching injury without compromising efficacy (Brosnan and Breeden 2013; Brosnan et al. 2013; Cox et al. 2017). Elmore et al (2015a, 2015b) found that the herbicide safener cloquintocet-mexyl (cloquintocet) applied in combination with topramezone reduces topramezone-induced creeping bentgrass (*Agrostis stolonifera* L.) injury and also increases creeping bentgrass biomass and PSII quantum yield. Elmore et al (2015b) suggests that creeping bentgrass tolerance to topramezone is influenced by cytochrome P450-catalyzed metabolism.

Creeping bentgrass (*A. stolonifera* L.) is a stoloniferous, cool-season turfgrass species, widely used on golf courses and other highly maintained turf areas in the United States and temperate regions of the world (Lyman et al. 2007; Christians 2011). Predominately used on golf course fairways, tees and greens, creeping bentgrass is best known for its tolerance to mowing heights as low as 3 mm (Christians 2011).

Topramezone has become an essential tool in turfgrass that may provide an alternative mode of action for weed control in creeping bentgrass. Previous research has reported moderate creeping bentgrass tolerance to topramezone can be influenced by different factors such as application rates, tank mixtures, growing season, soil/air temperature, precipitation, and other environmental conditions (Brosnan et al. 2013; Elmore et al. 2015a; Brewer et al. 2016; Johnston et al. 2016; Cox et al. 2017). The specifics of these factors remain poorly understood. Commonly turfgrass managers have used low topramezone application rates (<0.5 fl oz/a) and reapplications (weekly or biweekly) to controls crabgrass species (*Digitaria* spp.) and goosegrass [*Eleusine indica* (L.) Gaertn.]. This strategy aims to decreases bleaching injury in creeping bentgrass.

Although creeping bentgrass injury caused by topramezone has been frequently reported by turfgrass managers frequently, topramezone is simple straight forward herbicides to use and provide unique benefits to turfgrass weed control needs. We hypothesize that optimizing topramezone application timing, reducing rates, and tank-mix combinations can reduce turfgrass injury opening the door for possible future use of topramezone. An increased ability to use this

HPPD inhibitor can be an effective tool in turfgrass weed control with resistance to traditional chemistries becoming more prevalent in turfgrass.

Because there are few reports of safener efficacy of topramezone in creeping bentgrass and yet remain poorly understood which factors directly influence bleaching injury occurrence. The objectives of this research are to determine (i) the optimal timing of topramezone applications and (ii) the efficacy of topramezone tank-mixtures with paclobutrazol (Trimmit 2SC), Fe chelate (Sprint 330) or a turfgrass pigment (Sarge 2.0) to reduce bleaching injuries.

Materials and Methods

Research was conducted at the Auburn University Sports Surface Field Laboratory and Weed Science greenhouse in Auburn, Alabama from 2018 to 2019 to evaluate safening applications of topramezone in creeping bentgrass.

Field Trials. All field trials were conducted on Crenshaw creeping bentgrass putting green mowed at 0.3 cm daily throughout the experiments. The putting green's fertility, irrigation and disease control was maintained according to local extension service recommendations. The soil was a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) with a pH of 6.1 and 1.5% organic matter (OM).

In experiment 1, topramezone (Pylex^{MT} Herbicide 2.8C; BASF Corporation, Research Triangle Park, NC) was applied once in April, May, June, or July (Table 1). In experiment 2, three applications of tank-mix combinations of topramezone with paclobutrazol (Trimmit 2 SC, Syngenta Crop Protection, Greensboro, NC), Fe chelate (Sprint 330, BASF, Research Triangle Park, NC) or a green turf pigment (Sarge 2.0 Numerator Technologies, INC) were sequentially applied on a 21-day interval (Table 1). Foliar applications were delivered via a hand-held CO_2

pressurized sprayer equipped with TeeJet TP8002 (Spraying Systems Company, Wheaton, IL) flat fan nozzles spaced 25 cm apart and calibrated to deliver 280 L ha^{-1}.

The creeping bentgrass injury was rated at 4, 7, 10, 14, 18, and 21 days after application. Injury was evaluated relative to the nontreated on a 0 (no injury) to 100 (complete plant death) scale.

The experiments were arranged in randomized complete block designs. Data were subjected to ANOVA at the 0.05 probability level using SAS 9.4 (SAS Institute Inc., Cary, NC). Year by treatment interactions was detected for creeping bentgrass injury; therefore, the years are presented separately. The result of each replicate was expressed as mean and standard error of the mean (±SEM) for the four independent replicates. Graphs were plotted using Sigma Plot 10.0 (Systat Software Inc).

Greenhouse Trials. Two greenhouse experiments were conducted from November 2018 to February 2019 at the Auburn University Weed Science Greenhouse located on the main campus of Auburn University in Auburn, Alabama (32.35°N, 85.29°W). Greenhouse temperature was maintained between 30 and 25°C (day/night) with an average relative humidity of 68%. Single plugs (5 cm diameter) of creeping bentgrass were individually transplanted into plastic pots (10 cm diameter) filled a Marvyn sandy loam soil (fine-loamy, kaolinitic, thermic Typic Kanhapludults) with a pH of 6.5 and 1.1% organic matter. Pots were irrigated three times a day with overhead irrigation and fertilized (Miracle-Gro Water-Soluble All-Purpose Plant Food, Scotts Miracle-Gro Products INC., Maryville, OH) (28-8-16; ~6 kg N ha^{-1}) every two weeks to promote healthy growth.

Experiment 1 included: a nontreated control, topramezone applied alone, in a tank-mixture, three days before or three days after paclobutrazol, Fe chelate, or turfgrass pigment application (Table 2). Experiment 2 included: a nontreated control, topramezone applied at four different rates alone or in combination with paclobutrazol (Table 2). Paclobutrazol (102.7 g a.i. ha^{-1}) was applied 14 days before treatments on the topramezone plus paclobutrazol plots, to simulate a pre-existing PGR schedule.

Treatments applications were made with a CO_2 pressurized sprayer similar to field trials. Creeping bentgrass injury and photochemical efficiency (F_v/F_m) were measured at 4, 7, 9, 12, 15, 18, 21, and 25 DAT. Injury was evaluated relative to the nontreated on a 0 (no injury) to 100 (complete plant death) scale. F_v/F_m was assessed with a chlorophyll fluorometer designed for light-adapted yield (OS1-FL Portable | Opti-Sciences, Inc., Hudson, NH). F_v/F_m is the quantification of photosystem II efficiency. Photosystem II efficiency reduction can often be the first manifestation of stress on the leaf. (Maxwell and Johnson 2000; McElroy and Walker 2009b). The measurements were made approximately 0.5 cm from the creeping bentgrass leaves at midday (between 11:30 A.M. - 12:30 P.M. CST).

The experiment was arranged in a completely randomized factorial design and was replicated four times and repeated twice. Effects due to herbicide, application rate, and interactions were subjected to ANOVA at the 0.05 probability level using SAS 9.4 (SAS Institute Inc., Cary, NC). The F_v/F_m data were presented in percent relative to the nontreated. Negative % F_v/F_m indicates a decrease in photochemical efficiency. F_v/F_m and injury were expressed as mean and standard deviation of the mean (±SEM) of eight independent replicates. Graphs were plotted with Sigma Plot 10.0 (Systat Software Inc).

Results and Discussion

Significant year-by-treatment interactions were observed in application timing trials, application rates, and topramezone tank-mix combination in all field trials; therefore, data were analyzed and presented separately by year.

First field Trial. In 2018, one application of topramezone at 5.2, 10.4, 15.6, or 20.8 g a.i. ha^{-1} injured creeping bentgrass < 15% in April at 10 DAA (Figure 2 a). Maximum topramezone injury increased to >35 and 65% at 20.8 g a.i. ha^{-1} in May and July applications, respectively (Fig. 2 b, d). Interestingly, topramezone injured creeping < 25% in June (7 DAA) (Figure 2 c). This may have occurred due to the occurrence of high precipitation in the week preceding and following the application (Figure 1).

Overall, topramezone bleaching injury at rates tested in April, May, and June decreased after 14 DAA, without reducing creeping bentgrass quality. Only the July application seriously injured creeping bentgrass, reduced quality, and had slower recovery.

In 2019, mean bleaching injury 10 DAA was comparable in April and May, averaging <15%. Topramezone at 20.8 g a.i. ha^{-1} in June and 15.6, and 20.8 g a.i. ha^{-1} in July applications were the only treatments that caused unacceptable injury (> 20%) (Figure 2 g, h).

In field studies, Brewer et al (2016) found that single applications of topramezone at 36.8 g ha^{-1} had a predicted maximum creeping bentgrass injury of 70% 14 DAA. While Elmore et al (2015a) observed topramezone at the same rate injured creeping bentgrass approximately 26% in greenhouse studies at 14 DAA as well.

In the current study, most topramezone rates evaluated were less injurious in 2019 than in 2018, and this provided safer topramezone application for the turfgrass without affecting creeping bentgrass quality. A potential explanation is that reduced injury could be attributed to better creeping bentgrass health and less environmental stress in 2019, although weather data in both years did not show significant differences in temperature and relative humidity (Figure 1). In 2018, maximum creeping bentgrass injury occurred between 7 and 14 DAA, with injury decreasing after 14 DAA. In 2019, maximum injury occurred between 7 and 10 DAA, with injury decreasing after 10 DAA. It seems that favorable growing conditions in 2019 increased creeping bentgrass tolerance to applications of topramezone. According to the manufacturer, topramezone should be applied during favorable growing conditions for optimal turfgrass tolerance. Turfgrass under environmental stress is more susceptible to bleaching injury and turfgrass thinning (BASF 2015).

At the conclusion of the experiments, these data indicate that early-season application of topramezone is safe to creeping bentgrass at all rates used. July applications of topramezone injured creeping bentgrass the most significantly. This is likely due to the high temperatures that stressed the turfgrass during this period.

Second field Trial. In 2018, topramezone alone at 5.2 and 10.4 g a.i. ha^{-1} injured creeping bentgrass similarly or more compared to tank-mix combination with paclobutrazol (102.7 g a.i. ha^{-1}), Fe chelate (610.3 g a.i. ha^{-1}) or turfgrass pigment (1.68 kg ha^{-1}) after one, two or three sequential applications (Figure 3 a, b, c).

The tank mixture of topramezone plus paclobutrazol injured creeping bentgrass less than topramezone alone (Fig. 3 a). However, creeping bentgrass injury appeared later and remained visible in the field longer than injury from topramezone alone. We observed maximum creeping bentgrass injury by first topramezone application alone or plus Fe chelate or turfgrass pigment between 10 and 14 DAA. For topramezone plus paclobutrazol, the maximum injury occurred between 14 and 18 DAA.

In 2019, topramezone with or without paclobutrazol, Fe chelate or turfgrass pigment injured creeping bentgrass < 15% in the first and second sequential applications (Figure 3 d, e). Differences in creeping bentgrass injury was only observed after the third application and only topramezone (10.4 g a.i. ha^{-1}) plus paclobutrazol and topramezone (10.4 g a.i. ha^{-1}) plus Fe chelate reduced injury compared to topramezone (10.4 g ai ha^{-1}) alone (Figure 3 f).

In general, we observed at the, end of each 2-year field trial, sequential reapplications of topramezone with paclobutrazol and Fe chelate were the most efficient field treatments because of increased turfgrass quality and reduced topramezone injury (Figure 4). Topramezone plus paclobutrazol reduced topramezone injury more than Fe chelate.

These data and previous research show that maximum topramezone injury occurs between 7 and 14 days after application on new growth tissue in moderately tolerant turfgrass species (Brewer et al. 2016; Elmore et al. 2015a; Marble et al. 2018). We hypothesize that paclobutrazol safens application because maximum paclobutrazol efficacy occurs between 7 and 14 DAA as well, although this may change with growing degree-day during the growth phase, rates, and especially, grass species (Ferrell et al. 2003; Johnson 1992; Kreuser et al. 2018; March et al. 2013). Therefore, maximum turfgrass growth reduction by paclobutrazol results in reduced topramezone bleaching that primarily occurs in new growth tissue. Moreover, several benefits are attributed to the use of PGRs such as improve turfgrass color and density, as well as to enhance abiotic stress tolerance (Ervin and Zhang 2008).

Greenhouse Trials 1. Significant differences between experimental runs were not detected at (P > 0.05) for creeping bentgrass injury and F_v/F_m; therefore, data were pooled between experimental runs. The F_v/F_m values for nontreated creeping bentgrass ranged from 0.617 to 0.711 throughout the greenhouse trials. Topramezone alone at 20.8 g a.i. ha^{-1} reduced creeping bentgrass $F_v/F_m > 50\%$ at 7, 9, 12, 15, and 18 DAA (Fig. 5a). Paclobutrazol (102.7 g a.i. ha^{-1}), Fe chelate (610.3 g a.i. ha^{-1}) or turfgrass pigment (1.68 kg ha^{-1}) did not increase creeping bentgrass F_v/F_m in comparison to the nontreated check (Figure 5a).

In general, all topramezone treatments with paclobutrazol, Fe chelate or turfgrass pigment tank-mix combinations (Figure 5b), application three days before (Figure 5c), or application three days after (Fig. 5d) reduced creeping bentgrass F_v/F_m throughout the trials. Only tank mixtures of topramezone and paclobutrazol or topramezone applied three days after paclobutrazol application showed less F_v/F_m reduction compared to topramezone alone (Figure 5b, d).

Greenhouse Trials 2. Topramezone alone reduced creeping bentgrass F_v/F_m more than topramezone plus paclobutrazol at all application rates (Fig. 6). Topramezone alone at 5.2, 10.4, 20.8, and 41.6 g a.i. ha^{-1} reduced creeping bentgrass F_v/F_m 39, 48, 46, and 55% 9DAA, relative to nontreated plants, respectively. However, topramezone plus paclobutrazol reduced F_v/F_m 33, 38, 37, and 40%, respectively at the same time (Figure 6).

In general, an increase in bleaching injury caused by topramezone at 41.6 g a.i. ha^{-1} coincided with reductions in F_v/F_m. Similar reductions in F_v/F_m have been observed in bermudagrass leaf tissue treated with HPPD-inhibiting herbicides (Elmore et al. 2011b). However, topramezone alone at (5.2, 10.4, and 20.8 g a.i. ha^{-1}) injured creeping bentgrass < 20%, the photosynthetic efficiency was reduced by over 35%. Different results were observed for topramezone plus paclobutrazol treatments. Maximum F_v/F_m reduction occurred 9 DAA although in this same time was not seen creeping bentgrass injury by topramezone (Appendix B). Based on scientific literature, F_v/F_m is the quantification of the photosystem II efficiency, and photosystem II efficiency reduction can often be the first manifestation of stress on the leaf. (Maxwell and Johnson 2000; McElroy and Walker 2009b).

Research Implication. Results indicate that turfgrass managers opting for selective POST control of broadleaf or grassy weeds such as crabgrass species (*Digitaria* spp.) and goosegrass [*Eleusine indica* (L.) Gaertn.] in creeping bentgrass should primarily apply topramezone early in the growing season. Late-season topramezone applications are possible however, lower application rates are needed to avoid unacceptable levels of injury. Turfgrass health and quality should be assessed before applications because stressed turfgrass is more susceptible to bleaching injury. Environmental conditions may be related to different levels of topramezone injury in creeping bentgrass even at the same application rate (Brewer et al. 2016; Elmore et al. 2015a).

Topramezone could potentially be integrated into a selective weed control programs in creeping bentgrass with paclobutrazol, Fe chelate or green turfgrass pigment to reduce visual injury of applications. These products are labeled to improve overall turf quality. Healthier and more vigorous plants are more capable of withstanding environmental stress.

According to previous research, thylakoids that have an intact pigment inventory before herbicide treatment are not affected by the application of the bleaching herbicide. Typically, bleaching occurs only in newly formed leaves (Sandmann et al. 1991). Based on this research, we hypothesize that the integration of PGR and topramezone programs can be an additional tool that provides turf managers another option for weed control in creeping bentgrass putting greens. Additional work is underway to assess the topramezone safening potential of other PGRs on other turfgrass species.

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Table 1. Active ingredient and trade name, along with active ingredient rates utilized in the studies.

Treat.	Active Ingredient	Product	Rate g a.i. ha^{-1} *kg ha^{-1}
	Application timing – Field experiment 1		
1	Non-treated	-	-
2	Topramezone	Pylex	5.2
3	Topramezone	Pylex	10.4
4	Topramezone	Pylex	15.6
5	Topramezone	Pylex	20.8
	Tank-mix – Field experiment 2		
1	Non-treated	-	-
2	Topramezone	Pylex	5.2
3	Topramezone	Pylex	10.4
4	Topramezone + Paclobutrazol	Pylex + Trimmit	5.2 + 98.1
5	Topramezone + Fe chelate	Pylex + Trimmit	10.4 + 98.1
6	Topramezone + Fe chelate	Pylex + Sprint 330	5.2 + 610.3
7	Pylex + Pigment	Pylex + Sprint 330	10.4 + 610.3
8	Pylex + Pigment	Pylex + Sarge 2.0	5.2 + 1.68*
9	Topramezone + Triclopyr	Pylex + Sarge 2.0	10.4 + 1.68*

Table 2. Active ingredient and trade name, along with active ingredient rates utilized in the studies.

Treat.	Active Ingredient	Topramezone Application	Rate g a.i. ha^{-1} $^{*}kg\ ha^{-1}$
		Greenhouse – experiment 1	
1	Non-treated	-	-
2	Pylex	-	20.8
3	Paclobutrazol	-	102.7
4	Chelated Fe	-	610.3
5	Pigment	-	1.68*
6	Topramezone + Paclobutrazol	Tank mix	20.8 + 102.7
7	Topramezone + Fe chelate	Tank mix	20.8 + 610.3
8	Topramezone + Pigment	Tank mix	20.8 + 1.68*
9	Topramezone + Paclobutrazol	Before	20.8 +102.7
10	Topramezone + Fe chelate	Before	20.8 + 610.3
11	Topramezone + Pigment	Before	20.8 + 1.68*
12	Topramezone + Paclobutrazol	After	20.8 + 102.7
13	Topramezone + Fe chelate	After	20.8 + 610.3
14	Topramezone + Pigment	After	20.8 + 1.68*
		Greenhouse – experiment 2	
1	Non-treated	-	-
2	Topramezone	-	5.2
3	Topramezone	-	10.4
4	Topramezone	-	20.8
5	Topramezone	-	41.6
6	Topramezone + Paclobutrazol	Tank mix	5.2 +102.7
7	Topramezone + Paclobutrazol	Tank mix	10.4 + 102.7
8	Topramezone + Paclobutrazol	Tank mix	20.8 + 102.7
9	Topramezone + Paclobutrazol	Tank mix	41.6 + 102.7

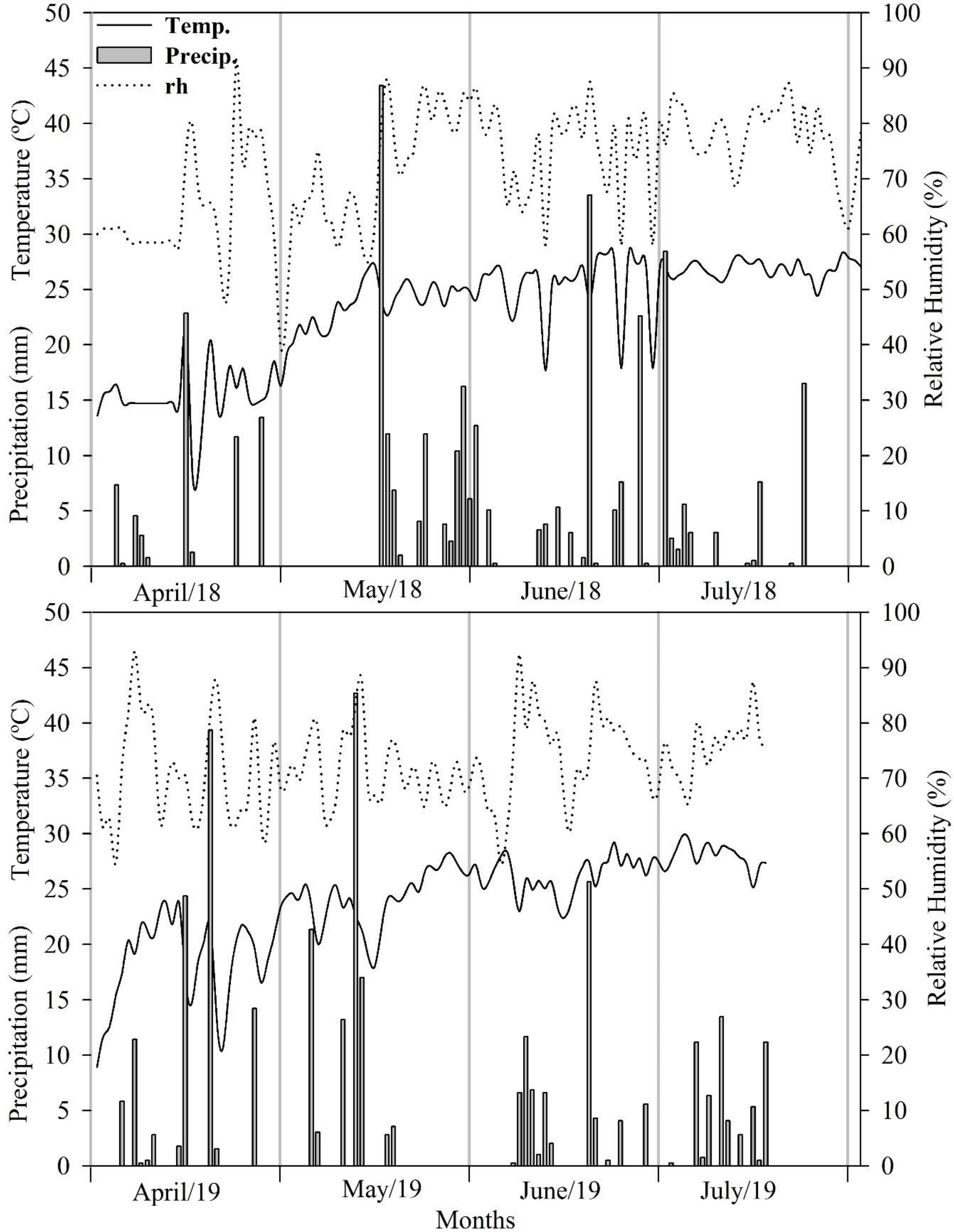

Figure 1. Precipitation (mm), temperature (°C), and relative humidity (%) during the months of April, May, June, and July in 2018 and 2019. Auburn University Turf Unit Station. *http://awis.aumesonet.com/weewx/public_html/AAFA1/*

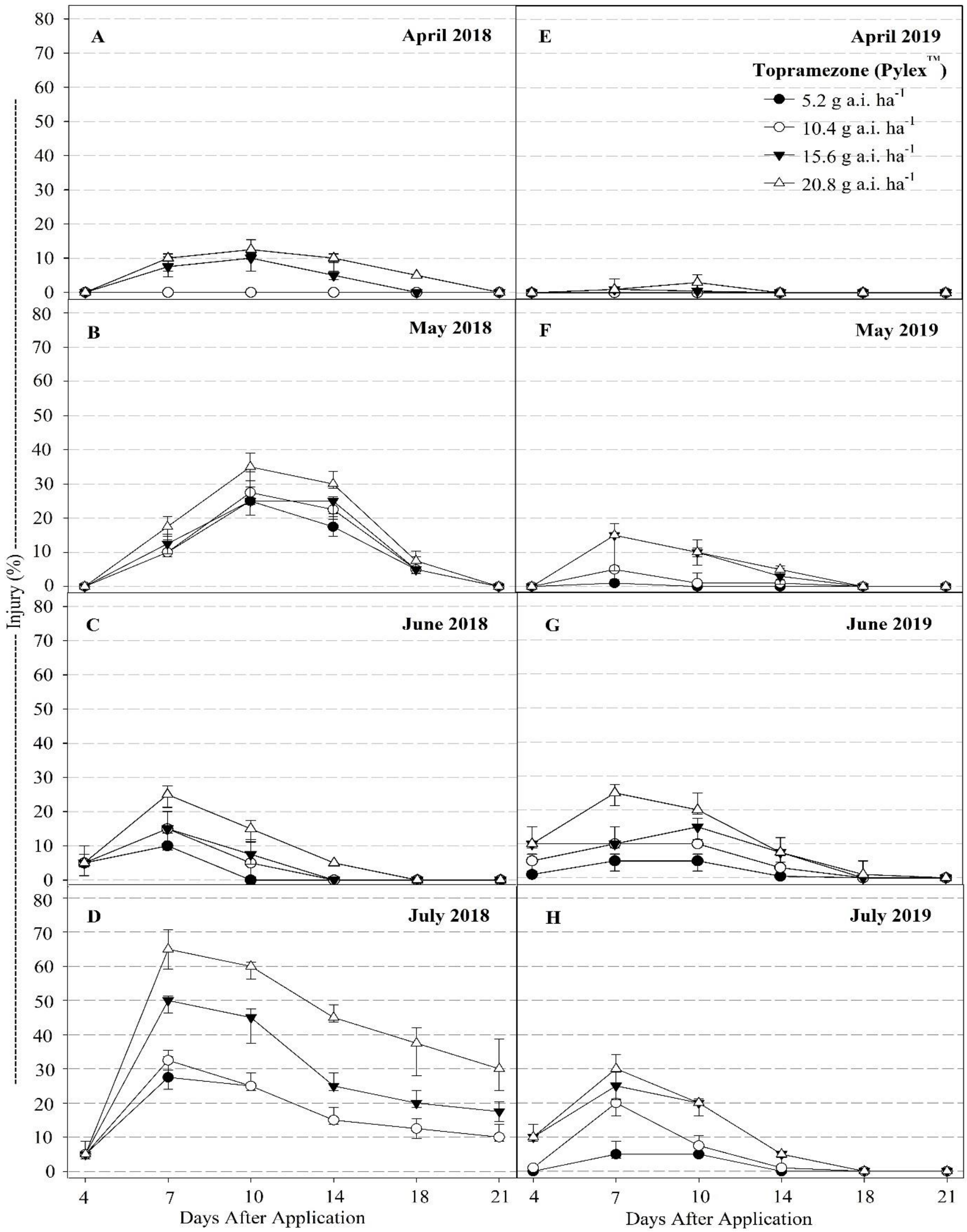

Figure 2. Visual estimation of creeping bentgrass foliar injury following topramezone application at four rates in April, May, June, and July 2018 and 2019. Error bars indicate standard errors of individual means, *n = 4*.

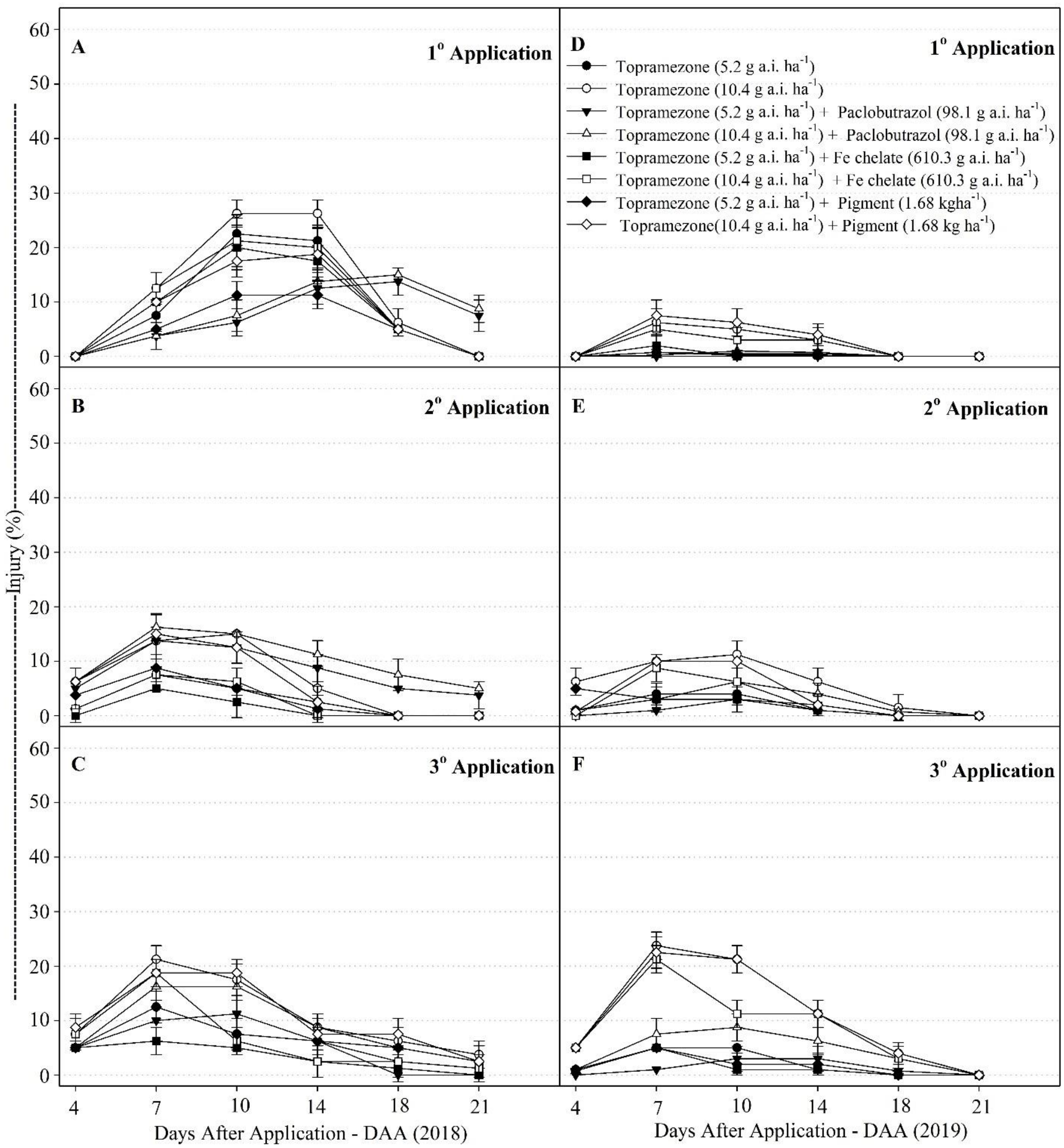

Figure 3. Visual estimation of creeping bentgrass foliar injury following sequential topramezone application alone and in combination with paclobutrazol, Fe chelate, or turfgrass pigment in 2018 (A, B, C) and 2019 (D, E, F). Error bars indicate standard errors of individual means, *n = 4*.

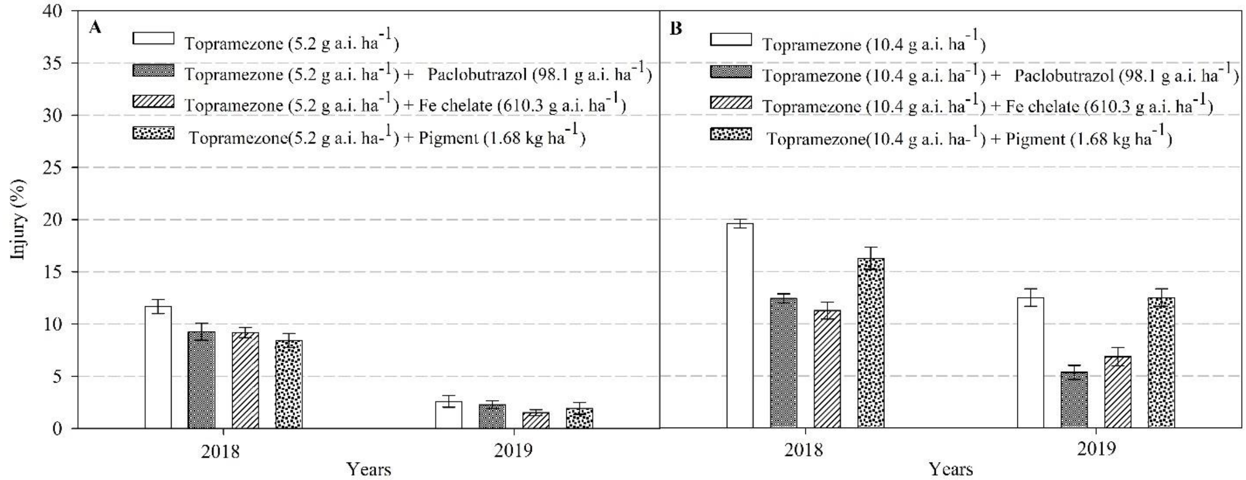

Figure 4. Average of visual estimation of creeping bentgrass foliar injury following three sequential topramezone applications at 5.2 g a.i. ha^{-1} (A) and 10.4 g a.i. ha^{-1} (B) every 21 days alone and in combination with paclobutrazol, Fe chelate, or turfgrass pigment in 2018 and 2019 (data collected10 days after each application). Error bars indicate standard errors of individual means, *n = 12*.

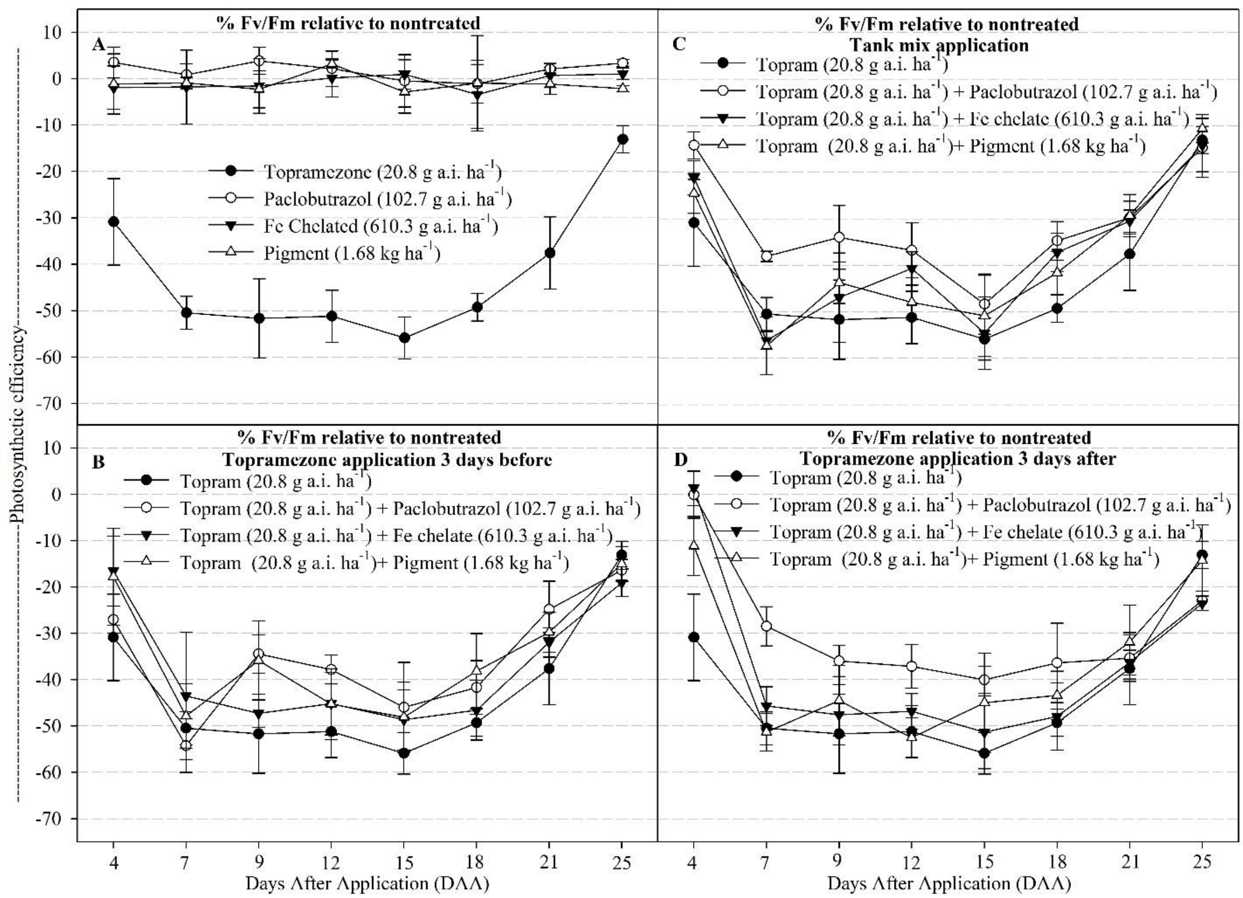

Figure 5. Photosynthetic efficiency of creeping bentgrass following topramezone, paclobutrazol, Fe chelate, or turfgrass pigment applied alone (A), topramezone application 3 days before (B), tank mix application (C), or topramezone application 3 days after (D). Error bars indicate standard errors of individual means, $n = 8$.

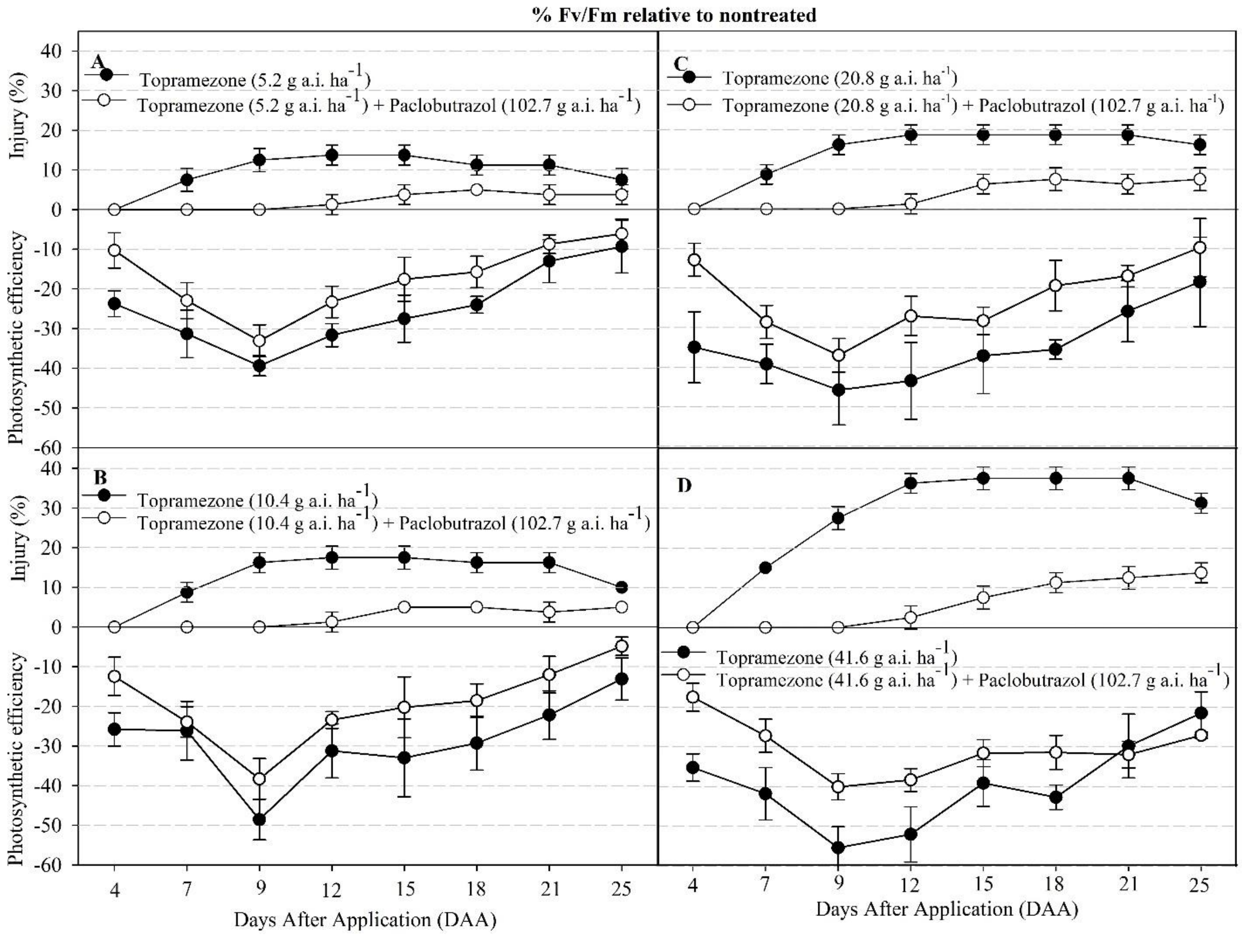

Figure 6. Injury and photosynthetic efficiency of creeping bentgrass following topramezone and paclobutrazol applied alone and tank mix application. Error bars indicate standard errors of individual means, *n = 8*.

APPENDIX A

Figure 2: Bermudagrass and seashore paspalum following topramezone and triclopyr application alone or tank-mixture. Total bermudagrass growth suppression was achieved with topramezone plus triclopyr.

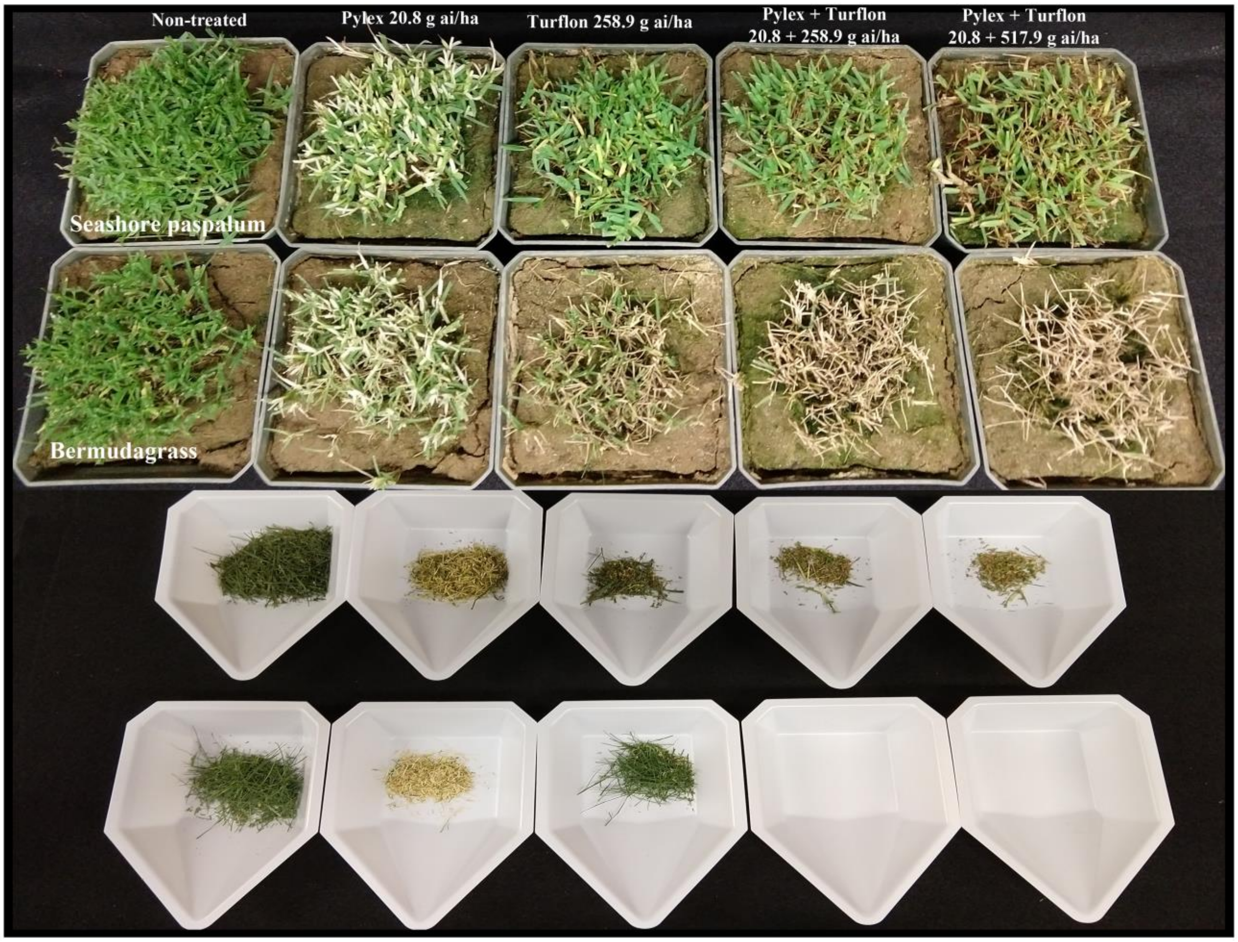

Figure 2: Bermudagrass control following topramezone tank-mixture with triclopyr. However, bermudagrass started regrowth by the regenerative underground organs.

APPENDIX B

Figure 1. Creeping bentgrass bleaching injury following topramezone application alone and in combination with paclobutrazol, Fe chelate or turfgrass pigment.

Figure 2. Creeping bentgrass bleaching injury following topramezone application alone and in combination with paclobutrazol. Paclobutrazol results in reduced bleaching caused by topramezone that occurs primarily in new growth tissues.