# Reducing Topramezone Injury to Bermudagrass Utilizing Micronutrients

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
December 12, 2020

Keywords: topramezone, HPPD, safener, iron, zinc, bleaching

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# Abstract

Bermudagrass (*Cynodon dactylon* L. Pers.) is one of the most common turfgrass species planted throughout the United States on golf courses, home lawns, and sports fields. Bermudagrass is able to tolerate a wide variety of environmental conditions; however, weeds can rapidly invade turfgrass areas weakened due to drought, disease, insect infestation, or mechanical damage. Grassy weeds such as goosegrass (*Eleusine indica* L. Gaertn.) and crabgrass (*Digitaria spp*.) often fill these voids and control can be problematic once established. Post-emergent control options of goosegrass and other grassy weeds continue to decline due to regulatory pressure and herbicide resistance. Previous research demonstrates that topramezone offers excellent control of goosegrass, crabgrass, and other weed species, and potentially could be a viable control option; however, injury to bermudagrass may be unacceptable. Topramezone safening research on bermudagrass is lacking. Multiple greenhouse, field, and growth chamber studies were performed from 2015 to 2020 in Auburn, AL. The objectives of these studies were to 1) evaluate various additives to determine whether bermudagrass bleaching injury was reduced; 2) determine whether iron application timings or different formulations of iron had an overall reduction effect; 3) assess other micronutrients for bleaching symptomology reduction; and 4) evaluate the effect that seasonal daylength and temperature have on chlorophyll and carotenoid production following topramezone application to bermudagrass. Data indicated that bermudagrass treated with a combination of topramezone and iron sulfate, chelated iron (DTPA), or zinc sulfate produced greatest reduction of bleaching symptomology out of all of additives tested. Furthermore, topramezone combinations that included nitrogen (N), whether alone or in combination with a nutrient product, often increased overall observed bleaching of bermudagrass. The addition of chelated iron, iron sulfate, or zinc sulfate safened the application of topramezone on bermudagrass. These findings should be communicated with turfgrass managers to help alleviate their concerns with use of this product, while also offering another mode of action to combat herbicide resistance.

# Acknowledgments

There are so many individuals that deserve recognition for their support and contributions to my research. Thanks go to my committee members, Dr. Elizabeth Guertal, Dr. David Han, Dr. Steve Li, Dr. Audrey Gamble, and Dr. Alvaro Sanz-Saez for your support and guidance through this process. All of you have always been so generous with your time and eager to help in any way that you can. My most sincere thanks go to my major advisor Dr. Guertal, who continues to push me to be a better writer and scientist each and every day. Your unwavering support and compassion truly shows through in each and every student's career that comes through the program, and I'm honored to have had you help guide me through this process.

I would also like to thank all of the individuals who helped me with countless hours of research, long road trips, photography, watering, potting, clipping, and sample analysis. Thanks go to William Head, Austin Brown, Maddie Benchwick, Cassidy Brown, David Lawrence, Clebson Goncalves, Frances Browne, Kati Price, Bill Bryce, Jim Harris, Jane Farr, and John Peppers. Thanks also go to all of the undergraduate students who have helped through the years behind the scenes. Without all of you, this research would not have been able to be completed. Thank you to all of my fellow graduate students in the department who were always there to lend a listening ear or help with questions. I really value all of the friendships that I have made with each and every one of you and wish all of you nothing but the best in your future careers.

I would like to express gratitude to all of my family and friends who have always supported me throughout these many years. I am so thankful to my beautiful daughter, Ellis, who came into the world two years ago, and has brought so much joy to me during that time. Each and every time you smile, it brightens my day and helps any cares or worries that I may have

disappear even for moment. I am so proud to be your daddy. To my wife, Katie, thank you for being my rock and the person that I can turn to during good times and bad. You are the most caring, loving, and generous person that I know and I am so grateful to be able to call you my wife. Thank you for your patience, love, and devotion during this process and for the sacrifices that you have made for our family. Ellis and I love you so much.

Table of Contents

# List of Tables

**Chapter 1.** Reducing Topramezone Injury to Bermudagrass Utilizing Chelated Iron and other Additives

**Chapter 2.** Impact of Iron Formulations on Topramezone Injury to Bermudagrass

**Chapter 3.** Micronutrient Inclusion to Reduce Bermudagrass Bleaching Associated with Topramezone

**Chapter 4.** Topramezone, Iron and Zinc Combinations on Hybrid Bermudagrass Physiological Responses

# List of Figures

**Chapter 3.** Micronutrient Inclusion to Reduce Bermudagrass Bleaching Associated with Topramezone

**Chapter 4.** Topramezone, Iron and Zinc Combinations on Hybrid Bermudagrass Physiological Responses

# List of Abbreviations

| | |
|---|---|
| AL | Alabama |
| DAT | Days after Treatment |
| DAIT | Days after Initial Treatment |
| Fe | Iron |
| FeEDTA | Ethylenediaminetetraacetic acid |
| FeEDDHA | Chelated iron sodium ferric ethylenediamine di-o-hydroxyphenyl-acetate |
| FeDTPA | Chelated iron diethylenetriaminepentaacetic acid |
| FW | Fresh Weight |
| g a.i. $ha^{-1}$ | Grams of active ingredient per hectare |
| HPPD | 4-hydroxyphenylpyruvate dioxygenase |
| kg $ha^{-1}$ | Kilograms per hectare |
| kg a.i. $ha^{-1}$ | Kilograms of active ingredient per hectare |
| mg $g^{-1}$ | Milligram per gram |
| MSO | Methylated seed oil |
| NDVI | Normalized difference vegetative index |
| RCBD | Randomized complete block design |
| ROS | Reactive oxygen species |

# Introduction

Turfgrass offers many benefits based on its aesthetic nature and wide range of usability. Due to its ability to tolerate an array of environmental conditions, bermudagrass is one of the most common turfgrass species planted on golf courses, home lawns, and sports fields in the southern United States (Beard 2001; Christians et al. 2017). As with any other turfgrass species, weed pressure can be problematic in bermudagrass. Infestations of grassy weeds such as goosegrass (*Eleusine indica* (L.) Gaertn), crabgrass (*Digitaria spp*.), and dallisgrass (*Paspalum dilatatum Poir.)* often result in a reduction of aesthetics and overall playability, safety concerns, as well as competition for available water and nutrients (Beard 2001). Healthy bermudagrass is the best defense against the encroachment of weeds, but turfgrass areas that have been damaged or thinned due to excessive traffic, drought, disease, insect infestation, or mechanical damage are prone to weed infestation (Christians et al. 2017; Holm et al. 1977).

Goosegrass is particularly troublesome in bermudagrass and is considered one of the worst grassy weeds of crops and turfgrasses in the world (Holm et al. 1977). Goosegrass continues to be a major problem for sports field and golf course managers, due to limited postemergence control options in bermudagrass (Busey 2001; Busey et al. 2009; Carrow and Petrovic 1992; Goatley et al. 2008; Johnson 1980; McCarty 1991; Turgeon 1980; Waddington 1992; Waddington and Baker 1965). No herbicides have been found that control mature goosegrass plants without also damaging bermudagrass (Busey 2004; Cox et al. 2017; Johnson 1975, 1980, 1996; McCarty 1991; Nishimoto and Murdoch 1999; Wiecko 2000).

Some of the most common active ingredients used for goosegrass control include monosodium methanearsonate (MSMA 6 Plus, Drexel Chemical Company, Memphis, TN), diclofop (Illoxan, Bayer Environmental Science, Research Triangle Park, NC), metribuzin

(Sencor, Bayer Environmental Science, Cary, NC), foramsulfuron (Revolver, Bayer Environmental Science, Cary, NC), sulfentrazone (Dismiss, FMC Corporation, Philadelphia, PA), and topramezone (Pylex, BASF Corporation, Research Triangle Park, NC)(Cox et al. 2017). MSMA was used to control multi-tillered goosegrass until 2009 when its use was banned in sports fields and heavily restricted on golf courses and sod production (Anonymous 2012; EPA 2006, 2013). Metribuzin was often mixed with MSMA to increase control of tillered goosegrass (Anonymous 2018). The registration for diclofop lapsed in 2015 at the request of the manufacturer. Combination products that contain foramsulfuron and/or sulfentrazone can control only seedling goosegrass (<2 to 3 tillers) (Anonymous 2013, Anonymous 2019).

Topramezone can control goosegrass, crabgrass, and dallisgrass at any growth stage (Anonymous 2018). Topramezone is a hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor which is associated with whitening or bleaching symptoms on the newest tissue in sensitive plant species (Brewer et al. 2017; Goddard et al. 2010; Grossmann and Ehrhardt 2007). Topramezone was initially registered in 2013 for bermudagrass and goosegrass removal from cool-season turfgrasses, but a supplemental label including bermudagrass was added in 2018, which focused on selective goosegrass control in bermudagrass at reduced rates (Anonymous 2018). Cox et al. (2017) showed reduced rates of topramezone on bermudagrass provides high levels of goosegrass control with only transient bleaching symptoms on the desired turfgrass. Bleaching is considered unacceptable on highly maintained turfgrasses due to its unnatural appearance and effect on overall aesthetics. This effect of application leads to some turfgrass managers being hesitant to use the product without a safening agent to eliminate or reduce the bleaching symptomology. If bleaching symptoms on bermudagrass could be eliminated or reduced, topramezone would offer an effective option for goosegrass and other grassy weed control in an otherwise diminishing list of herbicides.

## Herbicide Safeners

Ideal application of herbicides would involve applying the compound at the rate necessary for control of the target weed species, while not harming the desirable crop or turfgrass also present. In a cropping system, early injury associated with herbicide application often recovers and has little to no effect on the overall yield of the crop. In turfgrass systems, phytotoxicity associated with herbicide application is often discouraged due to its aesthetic nature (Yu et al. 2019a, 2019b). Selectively controlling the target weed species, while also rendering the desirable turf relatively unharmed is a challenge for turfgrass managers (Marble et al. 2015; Wolfe et al. 2016).

Compounds known as herbicide safeners have the ability to protect the crop or turfgrass from symptoms associated with herbicide application, without a reduction in weed control (Davies and Caseley 1999; Davies 2001). Herbicides, which are generally too injurious for certain crops, can be mixed with a safener and safely applied (Hatzios et al. 2004). This often expands the range of turfgrasses and crops that an herbicide can be applied to, while also offering another option to use in chemical rotations (Davies and Caseley 1999; Davies 2001; Elmore et al. 2015b; Elmore et al. 2016).

Turfgrasses inherently have enzymes which help to detoxify any foreign substances that enter into the plant (Elmore et al. 2016). The enzyme families which include cytochrome $P_{450}$ and glutathione transferase (GST) are responsible for the detoxification of most herbicides (Elmore et al. 2015a; Hatzios et al. 2004). These natural safeners are present in variable concentrations across different plant species (Kreuz et al. 1996). One notable example is creeping bentgrass (*Agrostis stolonifera* (L.). Creeping bentgrass has the ability to metabolize and detoxify the herbicide bispyribac-sodium, quicker than annual bluegrass (*Poa annua* L.) which leads to selective control of the weed species (McCullough et al. 2009). Although weed control is obtained, creeping bentgrass injury is still evident. This often causes the turfgrass manager to reduce the rate of herbicide in order to limit injury or use another product altogether (Dernoeden et al. 2008; Rutledge et al. 2010).

Another example is topramezone application to bermudagrass for goosegrass control. Topramezone offers excellent control at very low rates, but bleaching symptoms of the bermudagrass tissue may lead the superintendent to find another product to use. If a safening agent was used to reduce overall bleaching, while not affecting herbicide efficacy, it would make the product a more viable option. Several researchers have shown that combining triclopyr (Turflon Ester, Dow AgroSciences, Indianapolis, IN) with topramezone reduced the overall bleaching symptomology without a loss of herbicide efficacy, but increased levels of injury were also reported (Brosnan and Breeden 2013; Brosnan et al. 2013; Cox et al. 2017). Similarly, Lewis et al. (2010) reported that triclopyr reduced zoysiagrass (*Zoysia japonica* Steud.) injury when it was mixed with fluazifop-p-butyl. These findings are not surprising since previous research has shown some herbicides function as safeners when combined with other herbicides, which produced increased levels of enzymes and less overall injury (Edwards et al. 2000; Werck-Reichart et al. 2000).

Another study performed by Elmore et al. (2015a) reported that creeping bentgrass treated with topramezone and cloquintocet-mexyl in combination resulted in a reduction of injury when compared to bentgrass treated with topramezone alone. Their results showed that bentgrass treated with the combination had higher clipping yield as well as increased PSII quantum yield. They reported that the tolerance exhibited by creeping bentgrass was likely due to increased levels of cytochrome P450 which helped to increase detoxification of the topramezone (Elmore et al. 2015b).

Other sources of safening for herbicides including fertilizers and masking agents such as paints and pigments have also been reported. One strategy used by superintendents is to mask any injury that occurs following herbicide applications through the use of turfgrass pigments or paints (Pinnix 2014). Previous research has shown that the addition of chelated iron or ammonium sulfate has helped to reduce phytotoxicity when mixed with herbicides (Flessner et al. 2017; Johnson and Carrow 1995; Massey et al. 2006; McCarty 1991; McCullough and Hart 2009; Price 1983), but this effect has not been tested with topramezone.

## Micronutrients and their Safening Potential

When researching micronutrient usage in turfgrasses, it is evident that literature delving into the topic is lacking. There are arguably 17 elements that are essential for plant growth, which are divided into two groups: macronutrients and micronutrients (Salisbury and Ross 1992; Epstein and Bloom 2005; St. John et al. 2015). Macronutrients are those elements that are found in dry plant tissue in concentrations equal to or greater than 1000 mg $kg^{-1}$, while individual micronutrient concentrations are found at 100 mg $kg^{-1}$ or below (Salisbury and Ross 1992). When applied, this definition defines plant essential macronutrients as carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, calcium, magnesium, and sulfur, while micronutrients are defined as chlorine, iron, manganese, zinc, copper, boron, molybdenum, and nickel (St. John et al. 2015).

Evaluations of micronutrient use (other than Fe products) in turfgrass is sparse. The general perception is that native soils will typically provide sufficient micronutrients for turf growth. However, with newer engineered soils high in sand such as golf course putting greens and athletic fields, applications of micronutrients are often required to prevent deficiencies.

## Iron

Out of all of the micronutrients needed for plant growth, iron is required in the highest amounts within the plant. Iron plays many roles inside the plant, but arguably the most important ones include the formation of heme-proteins, iron-sulfur proteins, and synthesis of chlorophyll (Hopkins and Huner 2004). Important redox enzymes include the heme-containing cytochromes and non-heme iron-sulfur proteins involved in photosynthesis, nitrogen fixation, and respiration. One of these is the production of cytochrome P450, which is known to aid in herbicide detoxification and is heavily dependent on iron availability (Murgia et al. 2011). Another important role is its ability to transfer electrons by altering its moiety between the ferrous and ferric states (Salisbury and Ross 1992).

The involvement of iron in chlorophyll synthesis is relatively unclear, since it is not a direct part of the chlorophyll molecule (Salisbury and Ross 1992). No conclusive evidence has proven that the synthesis of chlorophyll depends on iron, but an overall requirement of iron for chloroplast construction and electron transport is better understood by research focused on iron deficiency. Direct studies of iron deficiencies often show that a lack of iron results in deterioration of the chloroplast structures and an overall loss of chlorophyll (Briat 2008). A yellowing appearance known as chlorosis will appear first on the youngest leaves of the plant due to the immobility of iron within the plant. Since iron is not transported from the older leaves to the newer leaves to counteract the deficiency symptoms, interveinal chlorosis on the newest leaves is a distinguishing characteristic (Hopkins and Huner 2004).

Iron is also crucial during the process of photosynthesis, because of its requirement during $CO_2$ fixation. This process ultimately leads to the production of $O_2$, which may produce reactive oxygen species (ROS) when reacted with iron (Ravet et al. 2009). In order to limit excess ROS from forming, iron homeostasis must be regulated between levels of deficiency and toxicity. Iron homeostasis is achieved through the release of phytosiderophores into the soil solution to aid in absorption of iron by the roots (Busch et al. 2008; Salisbury and Ross 1992). Following absorption, iron is trafficked throughout the plant to areas where it is needed, isolated and stored in the form of ferritin. These structures act as a storage facility for iron which aids in buffering potential for freely available iron within the plant (Briat et al. 2010). During periods of stress, ferritin releases iron which help the plant to cope with adverse conditions resulting from drought, elevated periods of light intensity, cold temperatures, oxidative stress, and disease (Busch et al. 2008).

Application of iron to turfgrass has been used to green turfgrass without promoting excessive growth (Carrow et al. 1988; Minner and Butler 1984; Yust et al. 1984). The most commonly applied Fe sources are Fe sulfate or Fe chelates (Cooper and Spokas 1991). Newer iron sources, such as Fe citrate, Fe glucoheptonate, and Fe humic acid materials have also been evaluated, although suitability as soil-applied sources varies widely with the product (Shaddox et al. 2018; 2019). In addition to color promotion, the use of Fe sulfate or Fe chelates as a safening material for herbicides has long been known. Researchers have found that iron can reduce symptoms of phytotoxicity associated with herbicide application on different turfgrass species (McCarty 1991; Price 1983; McCullough and Hart 2009; Johnson and Carrow 1995; Massey et al. 2006; Flessner et al. 2017), but topramezone in combination with iron was not tested.

## Boron

Boron is one micronutrient that is not fully understood based on its physiological roles within the plant. Boron is most often present as boric acid ($H_3BO_3$) in aqueous solutions (Hopkins and Huner 2004). Root uptake of boron is preferred in the dissociated form at or below a pH of 8. No evidence of involvement is known for boron with respect to specific enzymes or activators (Marschner 1995). Most knowledge is based solely on the results of what occurs when boron in deficient within the plant.

The largest proportion of borate within plant cells is found within the cell wall. Stable esters are formed when borate joins with saccharides which form derivatives of mannose and other polysaccharides (Hopkins and Huner 2004). Cells that are deficient in boron have shown abnormalities affecting the cell wall structure, which suggests that the structural integrity of cell walls relies heavily on boron availability.

Boron deficiency has also shown that boron plays a role in the elongation and division of cells (St. John et al. 2015). Elongation and division of cells deficient in boron often show inhibition in the plant roots. Boron deficiency results in the roots having a bushy or stubby appearance. In addition to the bushy and stubby appearance of roots, another symptom of boron deficiency is the enlarging of stems and shortening of internodes which often give plants a rosette appearance (Hopkins and Huner 2004). Although not occurring in turfgrass, one disorder which affects celery is known as “stem crack” where the stem becomes engorged and splits. Another disorder found in sugar beets is called “heart rot” due to the death of meristematic tissue.

No research involving boron as a safener in turfgrass has been shown to date. One previous study testing crop injury in sunflower (*Helianthus annuus* (L.)) reported that using boron in combination with the herbicides haloxyfop-methyl, sethoxydim, clethodim, and fluazifop-p-butyl did not cause any loss of weed control (Brighenti and Castro 2008). No antagonism of the herbicides or increase in crop injury were reported when combined with boron.

### Copper

Copper is generally found in well-aerated soils as the cupric ion form of $Cu^{2+}$. Copper performs primarily as a cofactor involving oxidative enzymes (Hopkins and Huner 2004). Some of the enzymes which are most affected by copper are plastocyanin (electron carrier in photosynthesis), cytochrome oxidase (respiration involving mitochondria), and ascorbic acid oxidase (Marschner 1995). Another extremely important enzyme that includes copper is superoxide dismutase (SOD), which aids in the detoxifying of ROS (St. John et al. 2015).

Very little research involving copper and herbicides has been performed. One study performed by Meybodi et al. (2011) showed that when bromoxynil + MCPA or 2,4-D +MCPA were used in combination with copper, there was no loss of herbicide efficacy on broadleaf weed control. Some turfgrass colorants and pigments will often include copper with the expressed intention of adding plant protection against environmental stresses. These studies have shown that when these materials are applied, Cu concentrations within the leaves increased substantially but no increases in overall quality or health were observed (McCarty et al. 2014).

### Manganese

Manganese is most often absorbed and transported throughout the plant as the cation $Mn^{2+}$ (Marschner 1995). One of the main roles that manganese plays within the plant is its function as an enzyme during the carbon cycle of respiration as dehydrogenase and decarboxylase enzymes (St. John et al. 2015). The most studied function of manganese involves its role in photosynthetic oxygen evolution as manganoprotein (Hopkins and Huner 2004). This manganese form is part of the complex which involves the evolution of oxygen and gain and loss of charges associated with the oxidation of water.

Deficiency of manganese is often dependent on the weather, conditions of the soil, crop species and can be extensive in certain areas. Some research involving manganese and herbicides have been conducted with varying results. A study performed by Bernards et al. (2005) showed that when glyphosate was applied with manganese, overall control of velvetleaf (*Abutilon theophrasti* Medik.), giant foxtail (*Setaria faberi* Herm.), and common lambsquarters were reduced, indicating antagonism of the mixture. Alternatively, the study mentioned before by Meybodi et al. (2011) showed no loss of efficacy of bromoxynil + MCPA or 2,4-D + MCPA when mixed with manganese. The differences reported in the two studies simply indicate that the presence of manganese has different effects on different herbicide formulations and modes of action.

**Zinc**

Uptake of zinc is mainly absorbed as the cation $Zn^{2+}$ (Marschner 1995). It is well known that zinc plays many roles as an activator in various enzymes including alcohol dehydrogenase, carbonic anhydrase, and superoxide dismutase (Hopkins and Huner 2004). It is also known that when zinc is deficient, plants often have shortened internode lengths and smaller leaves overall, indicating that metabolism of the auxin hormone indole-3-acetic acid is disturbed (St. John et al. 2015). This response is not fully understood, but zinc deficiency symptoms are often first observed as a reduction of auxin within the plant (Hopkins and Huner 2004). If zinc is applied to the plant, growth is quickly resumed (Marschner 1995).

Some research involving zinc and different herbicides have been previously studied. Most environmental stress relievers for turfgrass are compounds which include copper or zinc. When these products were applied, levels of zinc in the tissue increased, but no changes in plant health or overall quality were observed. (McCarty et al. 2014).

One research trial that tested multiple nutrients for herbicide safening potential was conducted by Patton et al. (2016). They researched various herbicide combinations in that included zinc, calcium, magnesium and manganese. Instead of finding safening of the herbicides, in most cases the nutrients antagonized their results with the exception of zinc. When zinc was added to the mixtures, no loss of herbicide efficacy was observed (Patton et al. 2016). These findings are similar to the ones reported by Roskamp et al. (2013) who tested multiple herbicides on horseweed (*Erigeron canadensis* (L.) Cronquist), kochia (*Kochia scoparia* (L.) Roth), redroot pigweed (*Amaranthus retroflexus* L.), and common lambsquarters (*Chenopodium album* L.). In all cases, when zinc was mixed with different herbicides in their trial, they reported no antagonistic effects (Roskamp et al. 2013). Additional work by Meybodi et al. (2011), showed no efficacy loss of broadleaf weed control when mixed with chelated combination products that included molybdenum, iron, zinc, copper, magnesium, cobalt, manganese, and nitrogen (Meybodi et al. 2011).

Research findings which contradict the previous work have also been reported. When zinc was mixed with glyphosate, weed control efficacy was reduced on a variety of grassy and broadleaf weeds (Huber 2007). Reductions in weed control were reported when glyphosate and zinc were combined and applied to barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.), browntop millet (*Urochloa ramose* (L.) Nguyen), palmer amaranth (*Amaranthus palmeri* S. Wats.), johnsongrass (*Sorghum halepense* (L.) Pers.), ivyleaf morningglory (*Ipomeae* *hederacea* Jacq.), and redroot pigweed (Scroggs et al. 2009). The authors attributed the antagonism of glyphosate to higher concentrations of Zn ions in solution, which was similar to previous work focusing on magnesium and calcium ions in hard water performed by Mueller et al. (2006).

The previous research has shown that zinc does have an effect on some herbicides which may act antagonistically or synergistically depending on the mode of action in question. Topramezone or any other HPPD inhibitors were not tested during any of these previous experiments, and safening research including topramezone should be researched.

## Research Objectives

1. Determine whether the addition of chelated iron, ammonium sulfate, triclopyr, green turf pigment, or green turf paint reduced the bleaching effect of topramezone injury on bermudagrass.
2. Examine the effects of Fe sources for their ability to safen topramezone use on bermudagrass.
3. Evaluate different rates of micronutrients in combination with topramezone to determine whether a reduction of bleaching or necrosis was observed.
4. Investigate photosynthesis response and pigment changes to hybrid bermudagrass subjected to two temperature/daylength regimes (seasons), following application of topramezone with or without FeDTPA/N, iron sulfate, and zinc sulfate.

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## Chapter 1.

# Reducing topramezone injury to bermudagrass utilizing chelated iron and other additives

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

# Chapter 1. Reducing topramezone injury to bermudagrass utilizing chelated iron and other additives[1]

## Abstract

Postemergence goosegrass and other grassy weed control in bermudagrass is problematic. Herbicides that can control goosegrass continue to decline due to regulatory pressure and herbicide resistance. Alternative herbicide options that offer effective control are needed. Previous research demonstrates that topramezone controls goosegrass, crabgrass, and other weed species; however, injury to bermudagrass may be unacceptable. The objective of this research was to evaluate safening potential of topramezone combinations with different additives on bermudagrass. Field trials were conducted at Auburn University during summer and fall from 2015 to 2018 and 2017 to 2018, respectively. Treatments included topramezone mixtures and methylated seed oil (MSO) applied in combination with five different additives including triclopyr, green turf pigment, green turf paint, ammonium sulfate, and chelated iron. Bermudagrass bleaching and necrosis symptoms were visually rated. Normalized difference vegetative index measurements and clipping yield were also collected. Results showed that topramezone plus chelated iron, as well as topramezone plus triclopyr, reduced bleaching potential the best; however, the combination of topramezone plus triclopyr resulted in necrosis

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[1] Published in Weed Technology (October 2020)

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that outweighed reductions in bleaching. Masking agents such as green turf paint and green turf pigment were ineffective in reducing injury when applied with topramezone. The combination of topramezone plus ammonium sulfate should be avoided due to the high level of necrosis. Topramezone-associated bleaching symptoms were transient and lasted 7 to 14 days on average. These data suggest that chelated iron added to topramezone and MSO mixtures acted as a safener on bermudagrass.

**Nomenclature:** Topramezone, [3-(4,5-dihydro-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone; triclopyr, 3,5,6-trichloro-2-pyridinyloxyacetic acid, butoxyethyl ester; crabgrass, *Digitaria spp.*; goosegrass, *Eleusine indica (L.) Gaertn.*; bermudagrass, *Cynodon dactylon (L.) Pers.*

**Key Words:** Bermudagrass, bleaching, chelated iron, safener, topramezone

## Introduction

Bermudagrass is one of the most common turfgrass species planted on golf courses, home lawns, and sports fields in the southern United States (Christians et al. 2017). Bermudagrass is able to tolerate a wide variety of environmental conditions. However, grassy weeds such as goosegrass, dallisgrass (*Paspalum dilatatum Poir.),* and crabgrass can rapidly invade turfgrass areas weakened due to drought, disease, shade, traffic, insect infestation, or mechanical damage (Holm et al. 1977). Infestations of grassy weeds often result in reduced playability, reduced aesthetics, safety issues, or water and nutrient competition for the desirable turfgrass (Beard 2001).

Postemergence weed control options in bermudagrass are limited, and none have been found that control mature goosegrass plants without damaging bermudagrass (Busey 2004; Cox et al. 2017; Johnson 1975, 1980, 1996; McCarty 1991; Nishimoto and Murdoch 1999; Wiecko 2000). Monosodium methanearsonate (MSMA 6 Plus, Drexel Chemical Company, Memphis, TN), diclofop (Illoxan, Bayer Environmental Science, Research Triangle Park, NC), metribuzin (Sencor, Bayer Environmental Science, Cary, NC), foramsulfuron (Revolver, Bayer Environmental Science, Cary, NC), sulfentrazone (Dismiss, FMC Corporation, Philadelphia, PA), and topramezone (Pylex, BASF Corporation, Research Triangle Park, NC) are the main active ingredients used for goosegrass control in bermudagrass (Cox et al. 2017). A common herbicide, MSMA, was used to control multi-tillered goosegrass until 2009 before its use was banned in sports fields and heavily restricted in sod production and on golf courses (EPA 2006, 2013). Metribuzin was often mixed with MSMA to increase tillered goosegrass control (Anonymous 2018). Diclofop registration expired in 2015 due to unprofitability. Products that contain foramsulfuron and/or sulfentrazone can control only seedling goosegrass (<2 to 3 tillers) (Anonymous 2013, Anonymous 2019).

Topramezone can control goosegrass, crabgrass, and dallisgrass at any growth stage (Anonymous 2018). Topramezone is a hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor which is associated with whitening or bleaching symptoms on the newest tissue in sensitive plant species (Brewer et al. 2017; Goddard et al. 2010; Grossmann and Ehrhardt 2007). Topramezone was initially registered in 2013 for bermudagrass and goosegrass removal from cool-season turfgrasses (Anonymous 2018). Cox et al. (2017) showed reduced rates of topramezone on bermudagrass provides high levels of goosegrass control with only transient bleaching symptoms on the desired turfgrass. Additional research showed that topramezone (6.15, 12.3 g ai $ha^{-1}$) mixtures combined with chelated iron (FeDTPA) rates, ranging from 0.1525 to 2.44 kg a.i. $ha^{-1}$, did not reduce topramezone efficacy for goosegrass control (Boyd et al. 2016). Complete control was achieved for the two goosegrass biotypes tested across all rates of topramezone and chelated iron (Boyd et al. 2016).

Bleaching is considered unacceptable due to aesthetic changes and striking symptomology following topramezone application. However, topramezone can control goosegrass and other grassy weeds at varying levels of maturity. Therefore, if bleaching symptoms on warm-season grasses could be eliminated or reduced, topramezone would offer an effective goosegrass and other grassy weed control option in an otherwise diminishing list of herbicides for this purpose.

Previous research has shown that the addition of chelated iron or ammonium sulfate has helped to reduce phytotoxicity when mixed with other herbicides (McCarty 1991; Price 1983), but this effect with topramezone has not been tested. Ammonium sulfate has been shown to increase the activity of herbicides when mixed with hard water (Flessner et al. 2017; O’Sullivan et al. 1981; Roskamp et al. 2013; Zollinger et al. 2010). Turf pigments and paints are often used to mask phytotoxicity or turf dormancy in bermudagrass, but no research has tested these agents for the masking of HPPD-application symptoms. Previous research has shown that bleaching symptoms are significantly reduced when topramezone is mixed with triclopyr (Turflon Ester, Dow AgroSciences, Indianapolis, IN), but injury is increased when this combination is applied to bermudagrass (Brosnan and Breeden 2013; Brosnan et al. 2014; Cox 2013; Cox et al. 2017). Thus, the objective of this study was to determine whether the addition of chelated iron, ammonium sulfate, triclopyr, green turf pigment, or green turf paint reduced the bleaching effect of topramezone injury on bermudagrass.

## Materials and Methods

A four-year study was conducted at the Auburn University Sports Surface Field Laboratory in Auburn, AL (32°34'N, 85°29'W). Specific location information is found in Table 1.

The bermudagrass cultivar used was 'Tifway' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy]. Treatment areas were maintained as a golf course fairway at a 2.5-cm height with scheduled mowing every three days during the trial. Clippings were returned during these mowing events. Normal fertilization practices for hybrid bermudagrass (36.6 kg N $ha^{-1}$ per growing month) were implemented on the site to maintain a healthy, dense turfgrass canopy. Prior to treatments, bermudagrass density was observed to be 100% with equal turfgrass quality across the trial area. Mowing was withheld for one day before and after applications. Any excess clippings left on the plot area were removed using a backpack blower. In order to maintain consistency across all plots, supplemental irrigation was applied on an as needed basis during the course of the study.

Treatments were made with a $CO_2$-pressurized backpack sprayer calibrated to deliver 280 L $ha^{-1}$ with a handheld, four-nozzle (TeeJet TP8002VS, TeeJet Spraying Systems, Roswell, GA) boom on 30.5-cm spacing. Applications were scheduled for periods when no rainfall was expected within 24 h of application timing. Topramezone was applied at 12.3 g ai $ha^{-1}$ alone and in combination with triclopyr at 35 g ai $ha^{-1}$, green turf pigment (Sarge 2.0, Numerator Technologies Inc., Sarasota, FL) at 1.75 kg ai $ha^{-1}$, green turf paint (Lesco Green Turf Paint, Site One Landscape Supply, Roswell, GA) at 8.3% v $v^{-1}$, ammonium sulfate (21-0-0, Hi-Yield Ammonium Sulfate, Bonham, TX ) at 1.68 kg N $ha^{-1}$, and chelated iron (Sprint 330, BASF, Research Triangle Park, NC) at 1.22 kg Fe $ha^{-1}$ (Table 2). Triclopyr at 35 g ai $ha^{-1}$ was also applied alone as an industry standard (Table 2). Triclopyr rate was based on previous research performed by Cox (2013), which illustrated significant reduction in bermudagrass bleaching over a range of 30 to 75 g ai $ha^{-1}$. All treatments included methylated seed oil (MSO, Alligare, Opelika, AL) at 0.5% v $v^{-1}$. A non-treated control was included for comparison. Trial area measured 12 by 12 m with individual experimental units of 1.5 by 3 m. Treatments were arranged in a randomized complete block design with 4 replications.

Single applications are reported by season and designated as either summer (June/July) or fall (September/October) based on initial application date (Table 1). Single applications occurring in summer and fall allowed for the evaluation of injury, which simulated what a turfgrass manager would encounter following one application of topramezone. Fall applications, while irregular throughout much of the US, are applied frequently in tropical areas such as southern FL and HI, where goosegrass perennates due to a lack of freezing temperatures (Holm et al. 1977; Uva et al. 1997). Sequential applications, often needed for control of certain weed species, were applied to evaluate the injury that might occur following multiple applications. For trials receiving a single application, all treatments were applied on the initial start date, with no additional applications of herbicide, fertilizer, or fungicide. For trials receiving two applications, a follow-up application was made 21 d after the initial treatment (DAIT). Sequential application intervals were based on findings from previous research (Askew 2012; Brewer et al. 2017; Brosnan and Breeden 2013; Cox et al. 2017).

Bermudagrass bleaching and necrosis were estimated visually utilizing a 0 to 100% scale, with 0% representing no bleaching and 100% being complete bleaching of all plant foliage. Necrosis percentages were defined as 0% representing no tissue death and 100% being complete tissue browning and death. Visually estimated bermudagrass symptoms of bleaching and necrosis were assessed 3, 7, 14, 21, and 28 DAIT for single applications, and 3, 7, 10, 14, 21, 24, 28, 35, 42, and 49 DAIT for sequential applications. For sequential applications, bleaching and necrosis data were collected immediately before the second (21 DAIT) application was made.

During the 2018 season, two additional measures, clipping yield and normalized difference vegetation index (NDVI), were added to further quantify treatment effects, and to provide preliminary data for future experiments. Turfgrass clipping yield was collected at 3-wk intervals by mowing a single pass down the center of each plot. A 50.8-cm wide TruCut (DOLPHIN Outdoor Power Equipment, Pompano Beach, FL) reel mower was used to mow a single swath through each plot at 2.5-cm height. During weeks when clippings were not collected, plots were mowed using a Toro Reelmaster 3100 set at 2.5 cm. All clippings were dried for 48 h at 60°C in a drying oven. Debris found within the bags was removed prior to weighing the dry matter.

Turfgrass canopy spectral NDVI data across treatments were recorded at 0, 7, 14, 21, 28, and 35 DAIT throughout 2018 using a handheld multispectral radiometer (Holland Scientific, Model ACS-430 Crop Circle) that was retrofitted to mount to a walk-behind golf trolley. The device was mounted at a stationary height of 46 cm above the turf canopy. Measurements of NDVI were calculated using the formula:

$$\text{NDVI}= \frac{R_{780} - R_{670}}{R_{780} + R_{670}} , \qquad [1]$$

where $R_{780}$ and $R_{670}$ were designated as the measured reflectance of near-infrared radiation (780 nm) and visible red radiation (670 nm) (Bremer et al. 2011; Trenholm et al. 1999). Radiometer measurements started at the center edge of each plot and an average of 50 readings per 1.5 m of linear travel were collected.

Trial data were separated by run within single or sequential applications. Single applications were separated further by season. Runs receiving a single application were analyzed together, while sequential applications were analyzed as a separate group (Table 1). Fixed effects were defined as treatment, DAT, season, and run, while repetitions were defined as random effects. Data were subjected to analysis of variance using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC, 27513) and means were separated using Tukey's HSD at $\alpha = 0.05$. Correlations of data were run using PROC CORR in SAS 9.4.

## Results and Discussion

**Bleaching.** For the most part, data followed similar trends for both the single and sequential applications, so data will be discussed together. The treatment-by-run-by-season interaction was not significant ($P = 0.403$). There was a significant treatment-by-run interaction (P <

0.0001) and treatment-by-season interaction ($P < 0.0001$) for the single applications grouping. Upon further inspection, data from runs within a season followed the same general trend. Once the runs were separated by season, no significant treatment-by-run interactions were observed and data were pooled across runs within a season for analysis. Each season was analyzed separately, and the treatment main effect was significant ($P < 0.0001$). For sequential applications, the treatment-by-run interaction ($P = 0.089$) and main effect of run were not significant ($P = 0.2709$), while the main effect of treatment ($P < 0.0001$) was significant. Bleaching data showed a significant correlation when compared with NDVI and necrosis. Data were pooled across all runs to present the overall treatment main effect.

During fall and summer seasons, symptoms of bleaching occurred from 7 until 21 DAIT (Table 3). The symptoms of bleaching were more pronounced during the fall season when compared with the summer season. Averaged across treatments, bermudagrass bleaching was 36.3% in fall compared with 21.5% in summer (Table 3). The sequential application trials also reflect these findings of bleaching occurring from 7 until 21 DAIT (Table 4). Following the 2nd application, bleaching symptoms returned with the first rating at 24 DAIT but dissipated completely by 42 DAIT.

As expected, triclopyr lacked bleaching symptomology since it is not an HPPD-inhibitor and will not be discussed further as a stand-alone treatment. At 7 DAIT in summer, topramezone plus ammonium sulfate bleached bermudagrass the most, and bleaching was equivalent to that found in the topramezone-only treatment. Adding green turf pigment, green turf paint, chelated iron, or triclopyr to the topramezone reduced bermudagrass injury, in comparison to topramezone plus ammonium sulfate treatment. None of the additives at 7 DAIT in summer reduced bleaching compared to that observed in the topramezone-only treatment

(Table 3). However, at 14 DAIT bermudagrass which received the topramezone plus chelated iron treatment did have reduced bleaching, as compared to that which received topramezone-alone or topramezone mixtures containing green pigment, green paint, or ammonium sulfate. In those treatments, bleaching continued to increase, while in the topramezone plus chelated iron treatment it was not changed from that at 7 DAIT (Table 3).

In the fall, at 7 DAIT the combination of topramezone and triclopyr had lowest bleaching, equal to that of the non-treated plots. Next lowest in bleaching was bermudagrass to which topramezone plus chelated iron had been applied. No other treatment created reductions in bleaching below that found in the topramezone-only treatment (Table 3). However, at 14 DAIT, the addition of green turf pigment, green turf paint, triclopyr, or chelated iron all reduced bermudagrass bleaching, as compared to topramezone alone. Fall application had greater bleaching when topramezone was applied alone, when compared to bermudagrass bleaching observed from summer applications. Differences due to additive were largely gone by 21 DAIT, except in the topramezone plus ammonium sulfate treatment, which had greater bleaching than almost every other treatment.

Similar results were observed in the sequential trials, as observed in the single application studies (Table 4). That is, greatest bleaching was often observed in bermudagrass receiving the topramezone plus ammonium sulfate treatment (highest at 5 of 10 rating dates). Use of chelated iron often reduced bermudagrass bleaching, when compared to application of topramezone alone (significant at 4 rating dates). The use of green pigment or paint was less useful for reducing bermudagrass bleaching, and neither of those treatments were effective (as compared to topramezone alone) after the second application (Table 4).

Our findings show that the addition of ammonium sulfate to topramezone sometimes had an additive effect on bermudagrass injury, which resulted in increased bleaching. Although bleaching was often higher when ammonium sulfate was added for the single applications, bleaching percentages were never significantly higher than those for bermudagrass with topramezone alone (Table 3). However, the trials with sequential applications did have greater damage when ammonium sulfate was added, and this was observed following the first application (7, 10, and 14 DAIT) and second application (24, 28, and 35 DAIT). Symptoms of HPPD inhibitor application appear first on the newest growth of the plant which would translate into higher levels of whitened tissue following a nitrogen application. Elmore et al. (2011) reported that when topramezone and mesotrione were applied to crabgrass plants treated with or without urea (49 kg $ha^{-1}$ or 0 kg $ha^{-1}$), crabgrass biomass was reduced more for plants treated with urea than with those not treated with urea. This could be expected since the addition of nitrogen effectively increases the plant's overall growth (Hull et al. 2005). However, N rates were low (1.7 kg $ha^{-1}$) in our study, which may have accounted for the differing effects of ammonium sulfate in our trials. Ammonium sulfate has also been shown to reduce antagonism associated with spray solutions containing hard water. Water quality reports from the city of Auburn, AL (2015-2018) show averages of water hardness ranging from 38-47 ppm, which the United States Geological Survey (USGS) categorizes as soft (0-60 ppm). Based on this data, hard water having an effect on the results of our trial can also be dismissed. The most likely cause of increased bleaching is an increase in herbicide uptake and translocation associated with the use of ammonium sulfate as a surfactant (Jordan et al. 1989; Maschhoff et al. 2000).

Attempts at masking the bleaching effect by combining topramezone with green turf paint or green turf pigment were typically short lived, and not effective. Over all trials the application of pigment reduced bermudagrass bleaching only once, when compared to topramezone alone. Application of paint was only effective three times, also compared to topramezone alone. Lack of response may be due to the regular mowing of bermudagrass maintained at fairway height. Paints and pigments are most effective when used on dormant turfgrass that has stopped growing for the winter (Pinnix 2014). Since these products were used on actively growing bermudagrass in the summer and fall, their effects were most likely removed via mowing within 10 to 14 DAIT.

The application of topramezone plus chelated iron was the only treatment that maintained an average bermudagrass bleaching percentage below the acceptable threshold of 20% for the entirety of the summer trials (Table 3). In the fall, the application of topramezone plus chelated iron to bermudagrass often yielded the lowest observed bleaching effects, but levels of bleaching were unacceptable, above the bleaching threshold of 20%. Similar results were observed in the sequential trials where bermudagrass to which topramezone plus chelated iron had been applied maintained an average bleaching percentage below 20%, for most dates. No previous research has used the combination of topramezone plus chelated iron to date, but these findings are similar to previous research performed using chelated iron and other herbicides to mask injury to desirable grasses (Flessner et al. 2017; Johnson and Carrow 1995; Massey et al. 2006; McCullough and Hart 2009). It is common practice for turfgrass managers to apply chelated iron to give turfgrasses a darker green appearance. The foliar application of iron allows the plant to increase the production of chlorophyll, which occurs quickly after application. Since iron is easily bound in the soil and rendered unavailable to the plant for uptake, foliar applications are most effective using either iron sulfate or a chelated iron product (Shaddox et al. 2018).

The application of topramezone plus triclopyr often reduced bermudagrass bleaching (compared to topramezone alone), with a significant reduction at four rating dates. However, the topramezone plus triclopyr treatment resulted in a different bleaching pattern compared to other treatments. Applications of topramezone alone resulted in a heavily bleached tissue, while the addition of triclopyr resulted in a bronzed appearance, rather than a fully white plant. There was also more of a sunken appearance to the plots treated with topramezone plus triclopyr when compared to the other treatments. These observations were also noted by Cox et al. (2017), where the application of topramezone plus triclopyr caused severe stunting and bronzing of the turfgrass with a reduction of whitening. Brosnan and Breeden (2013) found a higher level of persistent injury when topramezone plus triclopyr was applied, similar to our results.

The transient nature of the bleaching we observed in our trials corresponds with previous research documented by Elmore et al. (2011a, 2011b). Previous research has shown that triclopyr effectively stunts bermudagrass while topramezone whitens the newest growth (Brosnan and Breeden 2013; Brosnan et al. 2013; Cox et al. 2017; Goddard et al. 2010;). For this reason, bleaching in bermudagrass is effectively reduced; however, injury is increased and redistributed in the form of necrosis. These findings have also been documented by Brosnan et al. (2013) and Cox et al. (2017).

**Necrosis.** As was observed for bleaching, results were similar for both single and sequential applications, therefore data will be collectively discussed. The treatment-by-run-by-season interaction was not significant ($P = 0.325$). There was a significant treatment-by-run interaction ($P < 0.0001$) and treatment-by-season interaction ($P < 0.0001$) for the single application trials. As occurred with the bleaching response, all runs aligned with either the summer or fall season. Once runs were separated by season, no significant differences were observed for the interaction of run-by-treatment ($P = 0.412$), and data were pooled based on season. Each season was analyzed separately, and the treatment main effect was significant ($P < 0.0001$). For sequential applications, the treatment-by-run interaction ($P = 0.112$) and run main effect ($P = 0.174$) were not significant, while the treatment main effect was significant ($P < 0.0001$). Necrosis data showed a significant correlation when compared with NDVI, bleaching, and clipping yield. Data were pooled across all runs.

Bermudagrass necrosis was either not observed or slight at 3 DAIT, but by 7 DAIT differences due to treatment were apparent. Greatest necrosis was observed in bermudagrass receiving topramezone plus ammonium sulfate and topramezone plus triclopyr (Tables 5 and 6). Application of pigment or paint rarely reduced necrosis below that observed in bermudagrass receiving only topramezone (Table 5). Exceptions were the sequential applications, where the applications of pigment or paint did reduce necrosis, at 14, 21 (paint only), 28 (paint only), and 35 DAIT, compared to topramezone alone (Table 6).

Bermudagrass to which topramezone plus ammonium sulfate was applied often had greater necrosis; and this damage was often equal to that found in the topramezone plus triclopyr plots. These two treatments had the highest necrosis at 14 DAIT (summer, single application) and at almost every application date in the sequential trials (Tables 5 and 6). The application of chelated iron often reduced necrosis below that observed in the topramezone-only treatment. Of the twenty times that ratings were taken, the mixture of iron chelate plus topramezone reduced necrosis seven times when compared to that of the topramezone alone treatment. Those differences were more likely to be found in the sequential study (Table 6).

Our findings suggest that the combinations of topramezone plus ammonium sulfate and topramezone plus triclopyr should be avoided due to the high level of necrosis. Fall applications of topramezone plus triclopyr resulted in heavily damaged bermudagrass that struggled to recover following application. If the goal of the turfgrass manager is to remove bermudagrass from an area, these combinations would be most effective.

**Clipping Yield.** There was a significant treatment-by-season interaction (P = 0.0003) for clipping yield. The season and treatment main effects were analyzed individually by treatment (P < 0.0001) or season (P = 0.0006) (Table 7). Clipping yield showed significant correlations when compared with necrosis and NDVI.

The topramezone-only application was the only treatment to significantly reduce clipping yield, as compared to the non-treated control (7 DAIT, summer). At 35 DAIT, only the combination of topramezone plus triclopyr reduced yield, as compared to the non-treated control. Even though the symptoms of necrosis had subsided, the effects of canopy thinning were evident in this treatment.

More treatments affected clipping yield in the fall, with applications of topramezone, topramezone plus pigment, topramezone plus chelated iron, topramezone plus ammonium sulfate, and topramezone plus triclopyr all reducing clipping yield at 7 DAIT, as compared to the non-treated control. Some of these effects lasted for 35 days, with the application of topramezone, topramezone plus pigment, topramezone plus ammonium sulfate, and topramezone plus triclopyr all still reducing clipping yield, as compared to the non-treated control.

**Normalized Difference Vegetation Index.** None of the interactions were significant for NDVI (P = 0.2355-1.000). The treatment and season main effects were significant (P < 0.0001). Therefore, treatment main effects are presented separately for summer and fall season (Table 8). Correlations comparing NDVI with bleaching, necrosis, and clipping yield were all significant.

For the summer season, topramezone plus triclopyr had the lowest NDVI ratings amongst all treatments at 21, 28, and 35 DAIT. This effect persisted until the conclusion of the study, indicating that the bermudagrass sustained high levels of injury from which it struggled to recover (Table 8). The NDVI ratings demonstrated a general decrease in green tissue and recovery periods across trials. Bermudagrass following topramezone alone, topramezone plus green turf paint, and topramezone plus chelated iron treatments were quickest to recover.

During the fall trial, bermudagrass in the topramezone plus triclopyr treatment remained the most damaged 35 DAIT, as demonstrated by NDVI measurements (Table 8). Topramezone plus chelated iron had the highest NDVI ratings 28 and 35 DAIT, indicating bermudagrass recovery. It is apparent that the bermudagrass was heavily injured by late-season topramezone applications. Although bermudagrass appeared to fully recover from the bleaching and necrosis, NDVI measurements demonstrated reduced levels of overall plant health. These reduced levels are likely attributed to the late season slowing of growth, height reductions, and dormancy preparation. Research performed by Rana and Askew (2016), demonstrated that reductions in turfgrass height decreased NDVI measurements when applying methiozolin to bluegrass.

Previous findings have demonstrated that applications of topramezone to bermudagrass cause visual bleaching symptoms that are characterized by a reduction of chlorophyll and carotenoid pigments within the plant (Breeden et al. 2017; Brewer et al. 2017; Brosnan et al. 2011; Cox et al. 2017; Elmore et al. 2011a, 2011b). Across all of the previously mentioned trials, the symptoms associated with topramezone application were found to be transient in nature, which supports the findings of our study.

There are a multitude of factors which contribute to topramezone efficacy and bermudagrass injury; hence, the most appropriate topramezone program will vary across regions and turfgrass management practices. Future research should evaluate other iron formulations, micronutrients, and herbicides. Additional work should also include iron application timing and effects of soil moisture.

## Acknowledgments

The authors would like to thank David Lawrence and his crew at the Auburn Sports Surface Field Laboratory (Auburn, AL) for accommodating the multiple years of research. The authors would also like to thank Kathie Kalmowitz (BASF) for the donation of topramezone and chelated iron and continued support throughout the entirety of the research. No conflicts of interest have been declared.

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Table 1. Soil and site details across years for single and sequential application trials evaluating additives potential to reduce topramezone-associated bermudagrass bleaching in Auburn, AL.

| | | Application trial data | | | | | |
|---|---|---|---|---|---|---|---|
| Location | Soil type | Run name[a] | Soil pH | Initiation dates | Ending dates | Mean temperature °C | Season |
| Sports Surface Field Laboratory, Auburn University | Marvyn sandy loam (fine-loamy, kaolinitic, thermic, Typic Kanhapludult) | AU 1-15 | 6.1 | 7/17/2015 | 8/14/2015 | 26.7 | Summer |
| | | AU 1-16 | 6.2 | 7/18/2016 | 8/15/2016 | 27.2 | Summer |
| | | AU 2-16-1[b] | 6.0 | 6/27/2016 | 8/15/2016 | 27.2 | Summer |
| | | AU 2-16-2[b] | 6.2 | 7/18/2016 | 9/5/2016 | 26.7 | Summer |
| | | AU 1-17-1 | 6.3 | 7/14/2017 | 8/11/2017 | 25.6 | Summer |
| | | AU 1-17-2 | 5.9 | 9/29/2017 | 10/27/2017 | 18.9 | Fall |
| | | AU 2-17[b] | 6.1 | 7/5/2017 | 8/23/2017 | 25.6 | Summer |
| | | AU 1-18-1 | 6.0 | 7/26/2018 | 8/23/2018 | 25.6 | Summer |
| | | AU 1-18-2 | 6.1 | 10/2/2018 | 10/30/2018 | 19.4 | Fall |
| | | AU 2-18[b] | 6.1 | 7/9/2018 | 8/27/2018 | 25.6 | Summer |

[a] Run names defined as follows: AU x–y-z (x = 1 if single application, 2 if sequential application; y = year of experiment; z = run number occurring in that year (if applicable))

[b] Run names that tested sequential instead of single treatment applications.

Table 2. List of treatments and rates used for single and sequential applications for topramezone plus additive trial on bermudagrass in Auburn, AL from 2015 to 2018.

| Trade name[a] | Active ingredient | Herbicide rate | Additive rate[b] |
|---|---|---|---|
| | | g ai ha$^{-1}$ | |
| Non-treated Control | _ | - | - |
| Pylex® | Topramezone | 12.3 | - |
| Pylex® + Sarge 2.0 | Topramezone + pigment | 12.3 | 1.7 |
| Pylex® + Lesco Green Turf Paint | Topramezone + paint | 12.3 | 8.3 |
| Pylex® + Sprint® 330 | Topramezone + chelated iron | 12.3 | 1.2 |
| Pylex® + Hi-Yield Ammonium Sulfate | Topramezone + ammonium sulfate | 12.3 | 1.7 |
| Pylex® + Turflon® Ester | Topramezone + triclopyr | 12.3 | 35.0 |
| Turflon® Ester | Triclopyr | 35 | - |

[a] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v v$^{-1}$ in 1L bottles

[b] Pigment rate in L ha$^{-1}$, paint rate in % v v$^{-1}$, chelated iron and ammonium sulfate rate in kg nutrient ha$^{-1}$, triclopyr rate in g ai ha$^{-1}$.

Table 3. Visually-estimated bleaching percentage injury to Tifway bermudagrass based on single applications of topramezone plus additive treatments. Results shown are pooled over 4 experiments in summer grouping (2015 to 2018) and 2 experiments in fall grouping (2017 to 2018), Auburn, AL.

| | Bleaching[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Summer grouping | | | | | Fall grouping | | | | |
| Treatment[b] | 3 DAIT | 7 DAIT | 14 DAIT | 21 DAIT | 28 DAIT | 3 DAIT | 7 DAIT | 14 DAIT | 21 DAIT | 28 DAIT |
| | % | | | | | % | | | | |
| Topramezone | 1.3 ab | 25.8 ab | 53.3 a | 5 ab | 0.4 | 0 | 47.5 ab | 85 a | 27.5 bc | 0 |
| Topramezone/green turf pigment | 0.8 b | 20.4 b | 54.2 a | 4.6 ab | 0.8 | 0 | 37.5 bc | 57.5 b | 42.5 ab | 0 |
| Topramezone/green turf paint | 0.4 b | 14.6 bc | 40.4 ab | 1.7 ab | 0 | 0 | 45 ab | 55 b | 30 bc | 0 |
| Topramezone/chelated iron | 0 b | 13.3 bc | 14.6 c | 0 b | 0 | 0 | 25 c | 32.5 c | 15 cd | 0 |
| Topramezone/ammonium sulfate | 4.6 a | 36.7 a | 63.3 a | 11.7 a | 0.4 | 0 | 52.5 a | 80 a | 50 a | 0 |
| Topramezone/triclopyr | 1.7 ab | 17.9 b | 22.1 bc | 5.4 ab | 0 | 0 | 10 d | 52.5 b | 12.5 cd | 0 |
| Triclopyr | 0 b | 0 c | 0 c | 0 b | 0 | 0 | 0 d | 0 d | 0 d | 0 |
| LSD | 3.6 | 16.7 | 25.5 | 10.4 | - | - | 13.0 | 12.5 | 19.6 | - |
| *P* Value | 0.0036 | <0.0001 | <0.0001 | 0.016 | NS | NS | <0.0001 | <0.0001 | <0.0001 | NS |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

[c] Abbreviations: DAIT, days after initial treatment; NS, not significant.

Table 4. Visually-estimated bleaching percentage injury to Tifway bermudagrass based on sequential applications of topramezone plus additive treatments. Results shown are pooled from 4 experiments, 2016 to 2018, Auburn, AL.

| | Bleaching[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment[b] | 3 DAIT | 7 DAIT | 10 DAIT | 14 DAIT | 21 DAIT | 24 DAIT | 28 DAIT | 35 DAIT | 42 DAIT | 49 DAIT |
| | % | | | | | | | | | |
| Topramezone | 1.3 a | 49.7 b | 60 b | 18.4 b | 4.4 b | 15 b | 23.4 b | 9.4 b | 0 | 0 |
| Topramezone/green turf pigment | 0.9 ab | 46.6 b | 61.3 b | 12.8 bc | 2.5 bc | 18.8 ab | 17.2 bc | 6.9 b | 0 | 0 |
| Topramezone/green turf paint | 0.3 ab | 36.6 c | 40 c | 11.6 c | 1.7 bc | 8.8 bc | 15.3 cd | 5.8 bc | 0 | 0 |
| Topramezone/chelated iron | 0.6 ab | 25.6 c | 13.4 d | 3.1 cd | 0.6 c | 11.6 bc | 11.7 d | 2.2 bc | 0 | 0 |
| Topramezone/ammonium sulfate | 0.6 ab | 63.4 a | 76.9 a | 34.4 a | 11.3 a | 35.6 a | 44.1 a | 17.5 a | 0 | 0 |
| Topramezone/triclopyr | 1.3 a | 20 c | 35.6 c | 22.2 b | 9.1 a | 5.9 bc | 7.8 de | 6.9 bc | 0 | 0 |
| Triclopyr | 0 b | 0 d | 0 e | 0 d | 0 c | 0 c | 0 e | 0 c | 0 | 0 |
| LSD | 0.9 | 12.0 | 11.1 | 11.1 | 5.2 | 13.1 | 11.9 | 9.8 | - | - |
| *P* Value | 0.006 | <0.0001 | <0.0001 | <0.0001 | 0.004 | 0.0003 | <0.0001 | 0.0004 | NS | NS |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

[c] Abbreviations: DAIT, days after initial treatment; NS, not significant.

Second application followed ratings on 21 DAIT represented by black line on table

Table 5. Visually-estimated necrosis percentage injury to Tifway bermudagrass based on single applications of topramezone plus additive treatments. Results shown are pooled over 4 experiments in summer grouping (2015 to 2018), and 2 experiments in fall grouping (2017 to 2018), Auburn, AL.

| | Necrosis[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Summer grouping | | | | | Fall grouping | | | | |
| Treatment[b] | 3 DAIT[C] | 7 DAIT | 14 DAIT | 21 DAIT | 28 DAIT | 3 DAIT | 7 DAIT | 14 DAIT | 21 DAIT | 28 DAIT |
| | % | | | | | % | | | | |
| Topramezone | 0 | 11.3 ab | 29.4 b | 13.8 ab | 2.5 | 0 b | 0.6 ab | 53.8 ab | 48.8 bc | 29.4 b |
| Topramezone/green turf pigment | 0 | 11.9 ab | 25.6 bc | 7.5 b | 2.5 | 0 b | 0 b | 41.3 bc | 58.8 b | 20.6 b |
| Topramezone/green turf paint | 0 | 11.9 ab | 21.3 bc | 5.6 b | 1.3 | 0 b | 0 b | 48.8 ab | 38.8 bc | 16.9 b |
| Topramezone/chelated iron | 0 | 6.9 b | 8.1 cd | 1.3 b | 0.6 | 0 b | 0 b | 26.3 c | 27.5 cd | 12.5 b |
| Topramezone/ammonium sulfate | 0 | 18.8 a | 56.9 a | 35.6 a | 5 | 0 b | 8.8 ab | 63.8 a | 57.5 b | 28.8 b |
| Topramezone/triclopyr | 0 | 15 ab | 41.3 ab | 18.1 ab | 1.9 | 8.8 a | 10 ab | 65 a | 88.1 a | 72.5 a |
| Triclopyr | 0 | 7.5 b | 31.3 b | 0.6 b | 0 | 8.8 a | 11.3 a | 40 bc | 11.3 d | 11.9 b |
| LSD | - | 10.8 | 21.3 | 23.4 | 4.6 | 8.1 | 11.2 | 21.0 | 26.6 | 29.4 |
| *P* Value | NS | 0.022 | <0.0001 | 0.0006 | NS | 0.0003 | 0.0016 | <0.0001 | <0.0001 | <0.0001 |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% $v\ v^{-1}$ in 1L bottles.

[c] Abbreviations: DAIT, days after initial treatment; NS, not significant.

Table 6. Visually-estimated necrosis percentage injury to Tifway bermudagrass based on sequential applications of topramezone plus additive treatments. Results shown are pooled from 4 experiments from 2016 to 2018 in Auburn, AL.

| | Necrosis[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment[b] | 3 DAIT[c] | 7 DAIT | 10 DAIT | 14 DAIT | 21 DAIT | 24 DAIT | 28 DAIT | 35 DAIT | 42 DAIT | 49 DAIT |
| | --------------------------------------------------------- | | | | | % | --------------------------------------------------------- | | | |
| Topramezone | 0 | 15.9 b | 22.8 c | 28.1 b | 18.1 b | 19.7 b | 24.4 b | 22.2 bc | 3.1 b | 0 |
| Topramezone/green turf pigment | 0 | 12.5 b | 19.4 c | 18.4 c | 9.4 c | 15.9 bc | 16.6 b | 9.4 cd | 1.9 b | 0 |
| Topramezone/green turf paint | 0 | 13.1 b | 14.7 cd | 15 cd | 6.3 c | 7.5 c | 11.9 c | 7.2 cd | 1.6 b | 0.6 |
| Topramezone/chelated iron | 0 | 12.8 b | 10 d | 6.6 de | 4.4 c | 12.8 bc | 9.4 c | 4.4 d | 0.3 b | 0.3 |
| Topramezone/ammonium sulfate | 0 | 25.9 a | 38.1 a | 40.6 a | 27.2 a | 34.1 a | 36.3 a | 35.6 a | 10 a | 2.5 |
| Topramezone/triclopyr | 0 | 11.9 b | 29.7 b | 39.1 a | 32.2 a | 20.6 b | 22.5 b | 33.4 ab | 3.4 b | 0 |
| Triclopyr | 0 | 2.8 c | 20 c | 5 e | 3.4 c | 1.3 c | 0.6 c | 2.3 d | 1.3 b | 0.3 |
| | | | | | | | | | | |
| LSD | - | 6.8 | 9.4 | 8.0 | 8.9 | 13.3 | 9.9 | 12.1 | 2.7 | - |
| *P* Value | NS | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.025 | NS |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

[c] Abbreviations: DAIT, days after initial treatment; NS, not significant.

Second application followed ratings on 21 DAIT represented by black line on table

Table 7. Clipping yield of Tifway bermudagrass based on topramezone plus additive treatments in Auburn, AL in 2018.

| | Clipping yield[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Summer (2018) | | | | Fall (2018) | | | |
| Treatment[b] | 7 DAIT[c] | | 35 DAIT | | 7 DAIT | | 35 DAIT | |
| | ---------------g plot$^{-1}$----------------- | | | | ----------------g plot$^{-1}$-------------------- | | | |
| Control | 23.4 | a | 13.8 | ab | 15.2 | a | 16.2 | a |
| Topramezone | 3.7 | b | 5.4 | bc | 6.4 | b | 2.7 | d |
| Topramezone/green turf pigment | 14.7 | ab | 9.1 | abc | 4.8 | b | 7.9 | bcd |
| Topramezone/green turf paint | 7.1 | ab | 7.8 | abc | 14.1 | a | 12.6 | ab |
| Topramezone/chelated iron | 25.1 | a | 10.7 | abc | 7.5 | b | 17.6 | a |
| Topramezone/ammonium sulfate | 19.9 | ab | 15 | a | 6 | b | 9.4 | bc |
| Topramezone/triclopyr | 24.8 | a | 3.9 | c | 3.7 | b | 5 | cd |
| Triclopyr | 14.6 | ab | 10.4 | abc | 7.9 | b | 7.3 | bcd |
| | | | | | | | | |
| LSD | 11.9 | | 5.6 | | 3.8 | | 4.3 | |
| *P* Value | 0.006 | | 0.006 | | <0.0001 | | <0.0001 | |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)
[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v v$^{-1}$ in 1L bottles.
[c] Abbreviations: DAIT, days after initial treatment

Table 8. Spectral normalized difference vegetative index measurements of Tifway bermudagrass based on topramezone plus additive treatments in Auburn, AL in 2018.

| | NDVI[a,b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Summer (2018) | | | | | | Fall (2018) | | | | | |
| Treatment[b] | 0 DAIT | 7 DAIT | 14 DAIT | 21 DAIT | 28 DAIT | 35 DAIT | 0 DAIT | 7 DAIT | 14 DAIT | 21 DAIT | 28 DAIT | 35 DAIT |
| Control | 0.715 | 0.720 ab | 0.681 a | 0.692 a | 0.692 a | 0.703 a | 0.731 | 0.713 a | 0.700 a | 0.697 a | 0.648 a | 0.661 a |
| Topramezone | 0.706 | 0.712 ab | 0.543 b | 0.549 b | 0.658 bc | 0.711 a | 0.724 | 0.687 abc | 0.494 d | 0.408 d | 0.446 cd | 0.519 d |
| Topramezone/green turf pigment | 0.717 | 0.714 ab | 0.501 b | 0.450 d | 0.595 e | 0.710 a | 0.728 | 0.675 bcd | 0.461 d | 0.375 d | 0.417 de | 0.519 d |
| Topramezone/green turf paint | 0.723 | 0.692 ab | 0.509 b | 0.534 b | 0.632 cd | 0.705 a | 0.724 | 0.648 d | 0.458 d | 0.412 d | 0.467 c | 0.562 cd |
| Topramezone/chelated iron | 0.711 | 0.733 a | 0.533 b | 0.558 b | 0.644 c | 0.716 a | 0.727 | 0.700 ab | 0.573 c | 0.546 c | 0.539 b | 0.586 bc |
| Topramezone/ammonium sulfate | 0.707 | 0.715 ab | 0.502 b | 0.493 c | 0.606 de | 0.699 a | 0.730 | 0.663 cd | 0.448 d | 0.363 d | 0.403 e | 0.508 d |
| Topramezone/triclopyr | 0.712 | 0.678 b | 0.513 b | 0.383 e | 0.490 f | 0.636 b | 0.728 | 0.661 cd | 0.544 c | 0.408 d | 0.398 e | 0.434 e |
| Triclopyr | 0.711 | 0.708 ab | 0.653 a | 0.679 a | 0.684 ab | 0.710 a | 0.727 | 0.651 d | 0.617 b | 0.623 b | 0.618 a | 0.616 b |
| LSD | - | 0.03 | 0.03 | 0.03 | 0.02 | 0.01 | - | 0.03 | 0.04 | 0.04 | 0.03 | 0.04 |
| *P* Value | NS | 0.03 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS | 0.0002 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] Abbreviations: DAIT, days after initial treatment; NDVI, normalized difference vegetative index; NS, not significant.

[c] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

# Chapter 2.

## Impact of iron formulations on topramezone injury to bermudagrass

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

# Chapter 2. Impact of iron formulations on topramezone injury to bermudagrass

## Abstract

Goosegrass control options in bermudagrass are limited. Topramezone is one option that offers excellent control of mature goosegrass, but application to bermudagrass results in unacceptable symptoms of bleaching and necrosis observed with HPPD inhibitors. Previous research has shown that adding chelated iron reduced the phytotoxicity of topramezone without reducing the efficacy of the herbicide, resulting in safening when applied to bermudagrass. Our objective was to examine additional iron sources to determine if similar safening effects occur with other sources. Field trials were conducted in the summers of 2016 - 2018 (Auburn University) to determine if different iron sources safened topramezone on bermudagrass by reducing bleaching symptoms. Mixtures of topramezone and MSO were combined with 6 different commercial iron sources, including FeEDDHA, FeDTPA, iron citrate, $FeSO_4$, and a combination of iron oxide/sucrate/sulfate, some of which contained nitrogen. Bermudagrass necrosis and bleaching symptoms were visually rated on a 0 to 100% scale. Reflectance (NDVI) and clipping yield measurements were also collected. Application of FeDTPA and $FeSO_4$ reduced symptoms of bleaching and necrosis when applied with topramezone. Other treatments which contained nitrogen did not reduce injury but did reduce bermudagrass recovery time following the appearance of necrosis. Inclusion of small amounts of nitrogen often negated the safening effects

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[1] Accepted for publication in Weed Technology - (October 2020)

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of $FeSO_4$. The iron oxide/sucrate/sulfate product had no effect on bleaching or necrosis. Data suggests that iron source had a differential effect on bleaching and necrosis reduction when applied in combination with topramezone to bermudagrass. Overall, $FeSO_4$ and FeDTPA safened the topramezone the most on bermudagrass.

**Nomenclature:** Goosegrass, *Eleusine indica (L.) Gaertn.*; bermudagrass, *Cynodon dactylon (L.) Pers.;* topramezone, [3-(4,5-dihydro-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone; HPPD, 4-hydroxyphenylpyruvate dioxygenase inhibitors; MSO, methylated seed oil; FeEDDHA, chelated iron sodium ferric ethylenediamine di-o-hydroxyphenyl-acetate; FeDTPA, chelated iron diethylenetriaminepentaacetic acid; $FeSO_4$, ferrous sulfate
NDVI, normalized difference vegetative index.

**Key words:** Topramezone, bermudagrass, EDDHA, DTPA, ferrous sulfate, safener, turfgrass, bleaching, necrosis

## Introduction

Herbicide options for control of goosegrass (*Eleusine indica* (L.) Gaertn.) and other grassy weeds in bermudagrass (*Cynodon spp.*) are limited. Post emergent control options of goosegrass are extremely limited, with only a small number of effective herbicides available. One of these herbicides, topramezone (Pylex®, BASF Corporation, Research Triangle Park, NC), has shown excellent potential for control of mature goosegrass, even below labeled rates (Cox 2013). Unfortunately, topramezone is a hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, which when applied to bermudagrass leads to whitening or "bleaching" of the plant tissue (Anonymous 2018). This bleaching effect generally appears first on the newest tissue of any plants that are sensitive to the herbicide (Brewer et al. 2017; Goddard et al. 2010; Grossmann and Ehrhardt 2007). Although the bleaching effects are noticeable on the overall aesthetic of bermudagrass, effects are short-lived and transient in nature lasting on average 7 to 14 days (Boyd et al. 2016a; Brewer et al. 2017; Brosnan et al. 2011; Cox 2013; Cox et al. 2017; Elmore et al. 2011a, 2011b). No matter how well an herbicide performs, turfgrass managers and their clientele often focus on a high level of aesthetics and lengthy periods of bleaching or phytotoxicity on the desired grasses are discouraged.

Research performed at Auburn from 2015 to 2018 (Boyd et al. 2016a) indicated that the chelated iron diethylenetriamine pentaacetic acid (FeDTPA, Sprint®330, BASF, Research Triangle Park, NC), when mixed with topramezone, safened its use on bermudagrass by reducing overall bleaching and recovery time. Previous research also found that the mixture of chelated iron and other herbicides reduced the symptoms of phytotoxicity following application (McCarty 1991; Price 1983; McCullough and Hart 2009; Johnson and Carrow 1995; Massey et al. 2006; Flessner et al. 2017), but topramezone was not tested in these studies. Further research conducted in 2016, showed that FeDTPA combined with topramezone did not antagonize the efficacy of the herbicide for goosegrass control (Boyd et al. 2016b). Two goosegrass biotypes were tested and complete control was achieved across all rates of both FeDTPA and topramezone (Boyd et al. 2016b). Since topramezone combined with FeDTPA has the potential to control goosegrass and other grassy weeds at different stages of maturity without a loss of efficacy, it is important to determine whether other iron sources may offer additional safening options.

There are many different sources of iron used in plant nutrition, including sulfate, chelate, humate, oxide, and sucrate forms (Shaddox and Unruh 2018). Iron sulfate is a granular Fe source that is soluble in water and most effective when applied to the foliage via foliar spray (Shaddox et al. 2019; Yust et al. 1984). Due to its widespread use on golf courses and other high maintenance turfgrass areas, it is the most common Fe source used (Shaddox and Unruh 2018). Chelated iron comes in many forms, including ethylenediaminetetraacetic acid (EDTA), DTPA,  and ethylenediaminedi-o-hydroxyphenylacetic acid (EDDHA)(Shaddox and Unruh 2018; Shaddox et al. 2016). These formulations encapsulate the Fe ion within different organic complexes, which protect against oxidation in the soil once the material is applied. These materials offer turfgrass response when applied to the foliage or to the soil. Other chelated iron formulations include glucoheptanates, gluconates, and citrates. Shaddox et al. (2016) demonstrated that these materials often enhance turf color but have little effect on soil Fe availability. Iron oxides are another possible Fe source and are 99.5% water insoluble (Shaddox and Unruh 2018). No previous research has shown that this material offers turfgrass response. Last, iron sucrate is a powdered Fe oxide which is pelletized and manufactured with a sugar compound (Shaddox and Unruh 2018). The prills that are created disperse readily in water, but the Fe sucrate molecule remains insoluble.

Research indicates that only Fe chelate forms remain soluble in the soil for any length of time (Shaddox et al. 2016; 2019). When Fe sulfate, glucoheptonate, polysaccharide, humate, oxide or citrate was applied, the iron was insoluble within one hour of application. Only the forms of FeEDTA, FeDTPA, or FeEDDHA remained soluble in the soil at 21 days after application (Shaddox et al. 2019). Since some forms have limited residual solubility, this could affect the utility of various Fe forms as a safening agent for topramezone.

Given the plethora of iron sources used in turfgrass management, with clearly demonstrated differences in behavior (Shaddox et al. 2019), it is of interest to examine a variety of Fe sources for their ability to safen topramezone. Thus, the objective of this work was to examine the effects of Fe sources for their ability to safen topramezone use on bermudagrass.

## Materials and Methods

Studies were conducted over three one-month periods in August and September of 2016, 2017, and 2018. All studies were located at the Auburn University Turfgrass Unit in Auburn, AL (32°34'39.26"N, 85°29'55.32"W). For all trials, the soil type was a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult). Trials were 28 days in length, initiated in early or late August, and ended in September. In all, six trials were conducted, two each in 2016, 2017, and 2018. ‘Tifway’ hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy] was used for all studies. Research areas were mown at 2.5 cm to mimic conditions found on a golf course fairway. All mowing took place on three-day intervals and clippings were not removed. Research areas were observed to have a dense, healthy stand of bermudagrass and comparable turfgrass quality at the initiation of each trial. Mowing events were suspended for one day prior to and after each treatment application.

Foliar applied treatments were made using a handheld four nozzle boom fitted with TeeJet TP8002VS (TeeJet Spraying Systems, Roswell, GA) nozzles on 30.5 cm spacing and pressurized with a $CO_2$ backpack cylinder calibrated to deliver 280 L $ha^{-1}$. Topramezone was applied in combination with 6 different iron sources whose rates and vendor information are listed in Table 1. One of the products, iron oxide/sucrate/sulfate, is a granular source and was applied using a shaker can method over each designated plot in 3-4 directions. All treatments included methylated seed oil (MSO, Alligare, Opelika, AL) at 0.5% v $v^{-1}$. These treatments were compared to topramezone (12.3 g ai $ha^{-1}$) applied alone, as well as a non-treated check. All treatments were applied once on the initial start date, and no additional applications of herbicide, fertilizer, or fungicide were made during the course of the trial.

Visually estimated bermudagrass symptoms of bleaching and necrosis were assessed at 5, 10, 15, 21 and 28 days after initial treatment (DAT). Bleaching was measured on a 0-100% scale where 0% was defined as no observed bleaching and 100% as complete whitening of plant tissue. Necrosis was measured on a 0-100% scale where 0% was defined as no observed tissue death and 100% was total death and browning of the plant tissue. Measures of clipping yield and normalized difference vegetation index (NDVI) were added in 2018 to further quantify treatment effects. Clipping yield was determined by mowing a swath down the center of each plot at 7 and 21 DAT. A TruCut (DOLPHIN Outdoor Power Equipment, Pompano Beach, FL) reel mower which measured 50.8 cm in width was used at a height of 2.5 cm. Collected clippings were stored in paper bags and dried for a 48-hour period at 60°C in a drying oven. Any debris determined to not be turf clippings (twigs, leaves, etc.) were discarded prior to weighing. Plots were mowed with a Toro Reelmaster 3100 set at 2.5cm between the collection periods with all clippings being returned to the surface.

Spectral NDVI analysis was measured using a handheld multispectral radiometer (Holland Scientific, Model ACS-430 Crop Circle), which was attached to a 3 wheeled golf push cart and mounted at a stationary height of 46 cm parallel to the ground. Reflectance measurements were collected at 0, 5, 11, 21, 28, and 35 DAT throughout 2018. The NDVI measurements were calculated using the following formula

$$\mathrm{NDVI} = \frac{R_{780} - R_{670}}{R_{780} + R_{670}} \qquad [1]$$

where $R_{780}$ and $R_{670}$ are designated as the measured reflectance of near-infrared radiation (780 nm) and visible red radiation (670 nm) (Bremer et al. 2011; Trenholm et al. 1999). Reflectance readings were initiated at the center edge of each plot with approximately 50 readings being collected over the 1.5m distance of linear travel.

Trial area measured 6 by 12 m, with individual experimental units of 1.5 by 1.5 m in size. All trials had treatments arranged in a randomized complete block design with four replications. Data were analyzed within PROC GLM using Fisher's Protected LSD ($P < 0.05$) in SAS 9.4 (SAS Institute, Cary, NC, 27513). Correlations of data were run using PROC CORR in SAS 9.4.

## Results and Discussion

**Bleaching.** The treatment-by-run interaction ($P = 0.98$) and run main effect ($P = 0.45$) were found to not be significant, while the treatment main effect was highly significant ($P < 0.0001$). Data were pooled across all runs (Table 2). Correlations comparing bleaching with NDVI and clipping yield were significant.

Bleaching symptoms occurred at 5, 10, and 15 DAT and dissipated completely by the 21 DAT rating date for all trials (Table 2). At 5 DAT, the treatments of FeDTPA plus topramezone and $FeSO_4$ plus topramezone were the only applications which reduced bermudagrass bleaching percentage below that of topramezone applied alone. At 10 and 15 DAT, application of FeDTPA plus topramezone, FeEDDHA plus topramezone, and $FeSO_4$ plus topramezone were the only treatments that reduced bleaching, when compared to topramezone applied alone (Table 2). At 15 DAT, these three treatments had bermudagrass with bleaching comparable to the non-treated control, which was none. Highest levels of bleaching at 5, 10, and 15 DAT occurred on the iron sucrate/oxide/sulfate plus topramezone, topramezone-only, Fe citrate/urea plus topramezone, and $FeSO_4$/urea plus topramezone treatments. The iron oxide/sucrate/sulfate product (Ironite, Pennington, Madison, GA) is predominantly made up of iron oxide, which has been shown to be insoluble within 24 hours of soil application (Shaddox et al. 2019) and thus would not be available for plant uptake. Due to soil application of this granular material, it is understandable why the bleaching ratings were so similar between topramezone applied alone, and in combination with iron oxide/sucrate/sulfate.

Four of the sources contained N, which supplied 1.2, 4.6, 3.0, and 0.1 kg N $ha^{-1}$, respectively, when the FeEDDHA (Sprint®138, BASF, Research Triangle Park, NC), iron citrate (FeRROMEC®, PBI Gordon, Shawnee, KS), iron sulfate (FeRROMEC® AC, PBI Gordon, Shawnee, KS), and iron oxide/sucrate/sulfate (Ironite®) commercial sources were applied (Table 1). For summer application of N to bermudagrass these would be low rates of N, as compared to a typical application to a fairway of 24 – 49 kg N $ha^{-1}$ (intended to last about eight weeks).

Although no increase in plant bleaching was observed when N was included, as compared to topramezone alone, the decrease in bleaching typically seen with $FeSO_4$ was not observed in products which mixed $FeSO_4$ and other iron sources (Ironite) or $FeSO_4$ and N (FeRROMEC AC). When N was a part of the $FeSO_4$ (FeRROMEC AC), bleaching was never reduced compared to topramezone-only treatment, at any rating date. It appears that the inclusion of N at 3.0 kg ha$^{-1}$ did inhibit the ability of $FeSO_4$ to reduce bleaching. The only treatments which consistently reduced bleaching were the Fe chelates FeDTPA (all rating dates), FeEDDHA (10, 15 DAT) and $FeSO_4$ without N (all rating dates).

The only treatment which maintained bermudagrass with an average bleaching percentage below the acceptable injury threshold of 20% for the entirety of all trials was $FeSO_4$ plus topramezone (Table 2). The FeDTPA plus topramezone treatment created bermudagrass with bleaching above the threshold at 5 DAT, which then decreased below 20% at 10 and 15 DAT, and was then equal to bermudagrass bleaching observed in the $FeSO_4$ plus topramezone treatment. In this study, both FeDTPA and $FeSO_4$ repeatedly showed safening of bermudagrass, when used in conjunction with topramezone. Although not with topramezone, other researchers have shown similar results using mixtures of chelated iron and other herbicides to suppress or conceal injury of grasses (McCullough and Hart 2009; Johnson and Carrow 1995; Massey et al. 2006; Flessner et al. 2017).

**Necrosis.** The treatment-by-run interaction ($P = 0.827$) and run main effect ($P = 0.343$) were not significant, while the treatment main effect was significant ($P < 0.0001$). Data were pooled across all runs (Table 3). Correlations comparing necrosis with NDVI were found to be significant.

First symptoms of necrosis were observed at the 10 DAT rating date, across all trials (Table 4). At 10 DAT, the only bermudagrass that had necrosis comparable to that measured in the non-treated control was that to which FeDTPA plus topramezone had been applied. Most other bermudagrass had tissue damage greater than that observed in the non-treated control. All of the treatments, except for $FeSO_4$/urea plus topramezone, or iron oxide/sucrate/sulfate plus topramezone reduced overall necrosis at 10 DAT, when compared to topramezone applied alone (Table 3). At 15 DAT, all bermudagrass to which iron had been applied had reduced necrosis, when compared to that of topramezone applied alone. The topramezone-only and iron oxide/sucrate/sulfate plus topramezone were the only treatments that produced bermudagrass with injury above the 20% threshold (15 DAT). By 21 DAT, all of the treatments, excluding topramezone alone and iron oxide/sucrate/sulfate plus topramezone, had bermudagrass necrosis equal to that of the non-treated control. All signs of necrosis had fully dissipated by 28 DAT (Table 3).

As with reduced bleaching, the most effective treatments for reduced necrosis in bermudagrass were the Fe chelates (both FeEDDHA and FeDTPA) plus topramezone and $FeSO_4$ plus topramezone (Table 3). While the addition of iron citrate/urea also significantly reduced bermudagrass necrosis, the reduction was not as great as found in the FeDTPA or $FeSO_4$ treatments.

As with the bleaching data, the addition of N to the $FeSO_4$ appears to negate the effectiveness of $FeSO_4$ to safen topramezone. For example, at 10 DAT the $FeSO_4$/urea product (FeRROMEC AC) produced necrosis equal to that observed in the topramezone-only treatment. Previous trial work reported that the addition of N increased overall injury when mixed with herbicides (Jordan et al. 1989; Maschhoff et al. 2000), but additional necrosis was not observed in our study. The bermudagrass to which $FeSO_4$ (no N) plus topramezone was applied had significantly less necrosis. It is likely that the applied N, at 3.0 kg N $ha^{-1}$, was just enough to antagonistically affect the safening ability of the $FeSO_4$. This effect was short-lived, as at 15 and 21 DAT any product that had $FeSO_4$ included was equally effective at reducing necrosis.

**Clipping Yield.** The treatment-by-run interaction, main effect of run and treatment were all found to be non-significant across both collection dates in 2018. Since these data were found to be insignificant, the corresponding data will not be reported.

**Normalized Difference Vegetation Index.** The interaction of treatment-by-run, and main effects of treatment and run were significant ($P = 0.0124$, $P < 0.0001$, $P < 0.0001$), respectively. Corresponding data are shown in Table 4. Correlations comparing NDVI with bleaching and necrosis were found to be significant. Since NDVI results were statistically different for Run 1 and 2 of the 2018 trial periods, results will be discussed separately (Table 4).

For Run 1, minor differences in NDVI appeared at 5 DAT. Largest differences in NDVI response appeared at 11, 21, and 28 DAT, when all treatments receiving topramezone, with or without an iron additive, had lower NDVI readings than those measured in the non-treated control (Table 4). At 11 DAT, bermudagrass to which iron citrate/urea plus topramezone or $FeSO_4$ plus topramezone had a higher NDVI measurement than those measured when only topramezone was applied. The iron citrate/urea treatment had the highest N rate (4.6 kg N $ha^{-1}$) applied with that product, and a slight and short-term darkening in turf color would be expected. At 21 DAT, topramezone combinations mixed with FeDTPA, iron citrate/urea, $FeSO_4$/urea, and $FeSO_4$ all resulted in higher NDVI readings than observed in bermudagrass to which topramezone was applied alone. Bermudagrass with the highest NDVI readings was that which had been treated with $FeSO_4$ plus topramezone, or iron citrate/urea plus topramezone. By 28 DAT, all bermudagrass receiving topramezone had lower NDVI readings than those measured in the non-treated control, regardless of iron or N inclusion. By 35 DAT, no differences due to treatment were found (Table 4).

For Run 2, no differences were observed until 11 DAT (Table 4). All bermudagrass receiving topramezone, with or without an iron additive, had lower NDVI readings at 11, 21, and 28 DAT, when compared to NDVI readings from the non-treated control. By 11 DAT, bermudagrass that received topramezone combinations that included FeDTPA or $FeSO_4$ had higher NDVI readings than those found in the topramezone-only treatments. At 21 and 28 DAT, topramezone combinations with FeEDDHA, FeDTPA, $FeSO_4$/urea, and $FeSO_4$ all produced higher NDVI readings in bermudagrass than those measured when topramezone alone was applied (Table 4). By 35 DAT, application of $FeSO_4$ or FeDTPA plus topramezone were the only treatments which produced bermudagrass with higher NDVI readings. These two treatments produced bermudagrass with higher NDVI readings than those measured in the topramezone-only treatments on 5 of 8 rating dates (FeDTPA) and 6 of 8 rating dates ($FeSO_4$) across both runs (Table 4).

In order to better understand what these NDVI readings convey, it is important to discuss what the device is measuring. The spectral radiometer measures radiative and spectral differences associated with the red and near infrared (NIR) wavelengths, and it produces a number from -1 to +1 (Bremer et al. 2011). When measuring an area of turfgrass, values often range from 0.2-0.5 for bare ground, varying stages of dormancy, moisture content, and injury or necrosis, while values of 0.5 – 0.9 indicate turfgrass with varying levels of plant health and chlorophyll content (Bell et al. 2004; Bremer et al. 2011; Stiegler et al. 2005; Goodin and Henebry 1998). The red wavelength is part of the visible light spectrum and is influenced by the presence of chlorophyll (Gausman 1977). A high chlorophyll content in the plant tissue translates to a lower reflectance value of the red wavelength and a higher reflectance value of

NIR, which results in a higher NDVI value. Since more of the red-light spectrum is absorbed, the plant tissue appears green to the human eye. Alternatively, a low chlorophyll content will reflect higher levels of the red-light spectrum and lower levels of NIR, which results in a lower NDVI value and a brown or yellowish appearance to the observer (Karcher and Richardson 2003; Bremer et al. 2011; Trenholm et al. 1999).

Previous research has determined that topramezone application to bermudagrass results in visually unacceptable injury due to the destruction of carotenoid and chlorophyll pigments within the leaf tissue (Cox 2013; Elmore et al. 2011a; Elmore et al. 2011b; Brosnan et al. 2011; Cox et al. 2017). These findings corroborate the results of our trial and help to explain the differences observed in our work. Previous trials have all reported that the symptoms associated with topramezone are transient and short-lived, which our findings also substantiate. Overall, our results indicated that inclusion of $FeSO_4$ or FeDTPA both offered superior safening when applied in combination with topramezone on bermudagrass. The iron citrate/urea and $FeSO_4$/urea products both aided in recovery, but higher initial injury should be expected. The iron oxide/sucrate/sulfate product was ineffective in reducing injury associated with topramezone application when applied concurrently with the herbicide. Turfgrass managers should be aware of the potentially altering effects that nitrogen has on bermudagrass treated with topramezone, and make a conscious effort to closely monitor the products they choose to combine and apply.

## Acknowledgments

The authors would like to thank Kathie Kalmowitz and her team at BASF for the donation of topramezone and Sprint®138 and 330 products. Without their continued support this research would not have been possible. David Lawrence and his crew at the Auburn University Turfgrass Research Unit in Auburn supplied the turfgrass areas needed for multiple years of research. Also, thanks go to Jeff Buckner at Crown Industries, Alan Estes and Scott Wanzor at PBI Gordon for their donation of products for this research. No conflicts of interest have been declared.

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Table 1. List of treatments and rates used for topramezone + iron sources on bermudagrass in Auburn, AL from 2016-2018.

| Active ingredient | Trade name[a] | Manufacturer | Topramezone rate (g a.i. $ha^{-1}$) | Product rate[b] | Guaranteed analysis %-N-$P_2O_5$-$K_2O$-Fe | N applied (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|
| Topramezone | Pylex® | BASF<br>Research Triangle Park, NC | 12.3 | --- | --- | --- |
| FeEDDHA<br>Topramezone | Sprint® 138 | BASF<br>Research Triangle Park, NC | 12.3 | 2.03 kg $ha^{-1}$ | 4-0-0-6 | 1.2 |
| FeDTPA<br>Topramezone | Sprint® 330[c] | BASF<br>Research Triangle Park, NC | 12.3 | 1.22 kg $ha^{-1}$ | 0-0-0-10 | 0.0 |
| Iron citrate<br>Urea<br>Topramezone | FeRROMEC® | PBI Gordon<br>Shawnee, KS | 12.3 | 30.5 L $ha^{-1}$ | 15-0-0-6 | 4.6 |
| Ferrous sulfate<br>Urea<br>Topramezone | FeRROMEC® AC | PBI Gordon<br>Shawnee, KS | 12.3 | 20.3 L $ha^{-1}$ | 15-0-0-6 | 3.0 |
| Iron oxide<br>Iron sucrate<br>Ferrous sulfate<br>Topramezone | Ironite® | Pennington<br>Madison, GA | 12.3 | 6.1 kg $ha^{-1}$ | 1-0-1-20 | 0.1 |
| Ferrous sulfate<br>Topramezone | Ferrous Sulfate | Crown Technology Inc.<br>Indianapolis, IN | 12.3 | 6.1 kg $ha^{-1}$ | 0-0-0-20 | 0.0 |

[a] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

[b] Product rates based on the Fe application rate of 1.2 kg a.i. $ha^{-1}$

[c] Earlier formulation of this product was used for experiment. Product has recently been reformulated to include 7% nitrogen.

EDDHA - Sodium ferric ethylenediamine di-o-hydroxyphenyl-acetate; DTPA – Diethylenetriamine pentaacetic acid

Table 2. Visual percentage of bleaching injury to Tifway bermudagrass based on single applications of various iron + topramezone treatments. Results shown are pooled over 6 experiments conducted in Auburn, AL (2016-2018).

| | Bleaching (%)[a] | | | |
|---|---|---|---|---|
| Treatment[b] | 5 DAT | 10 DAT | 15 DAT | 21 DAT |
| Non-treated Control | 0.0 e | 0.0 d | 0.0 c | 0.0 |
| Topramezone | 45.8 ab | 49.2 a | 12.5 a | 0.0 |
| FeEDDHA<br>Topramezone | 35.0 bc | 31.7 b | 3.5 bc | 0.0 |
| FeDTPA<br>Topramezone | 26.7 cd | 15.4 c | 1.1 c | 0.0 |
| Iron citrate<br>Urea<br>Topramezone | 45.0 ab | 42.5 ab | 8.3 ab | 0.0 |
| Ferrous sulfate<br>Urea<br>Topramezone | 48.3 ab | 51.7 a | 10.4 a | 0.0 |
| Iron oxide<br>Iron sucrate<br>Ferrous sulfate<br>Topramezone | 54.2 a | 51.7 a | 10.0 a | 0.0 |
| Ferrous sulfate<br>Topramezone | 17.1 d | 14.2 c | 2.8 bc | 0.0 |
| LSD | 16.0 | 10.9 | 6.4 | - |
| *P* Value | <0.0001 | <0.0001 | <0.0001 | NS |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

DAT = days after initial treatment; LSD = least significant difference

Table 3. Visual percentage of necrosis injury to Tifway bermudagrass based on single applications of various iron + topramezone treatments. Results shown are pooled over 6 experiments conducted in Auburn, AL (2016-2018).

| | Necrosis (%)[a] | | | | |
|---|---|---|---|---|---|
| Treatment[b] | 5 DAT | 10 DAT | 15 DAT | 21 DAT | 28 DAT |
| Non-treated Control | 0.0 | 0.0 f | 0.0 c | 0.0 b | 0.0 |
| Topramezone | 0.0 | 39.2 a | 34.2 a | 4.2 a | 0.0 |
| FeEDDHA<br>Topramezone | 0.0 | 19.2 cd | 2.9 c | 0.0 b | 0.0 |
| FeDTPA<br>Topramezone | 0.0 | 6.3 ef | 0.8 c | 0.0 b | 0.0 |
| Iron Citrate<br>Urea<br>Topramezone | 0.0 | 27.5 bc | 7.1 c | 0.4 b | 0.0 |
| Ferrous Sulfate<br>Urea<br>Topramezone | 0.0 | 31.7 ab | 8.8 c | 0.4 b | 0.0 |
| Iron Oxide<br>Iron Sucrate<br>Ferrous Sulfate<br>Topramezone | 0.0 | 33.3 ab | 22.9 b | 2.5 ab | 0.0 |
| Ferrous Sulfate<br>Topramezone | 0.0 | 11.7 de | 2.3 c | 0.0 b | 0.0 |
| LSD | - | 11.3 | 8.9 | 3.7 | - |
| *P* Value | NS | <0.0001 | <0.0001 | 0.0033 | NS |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% $v\ v^{-1}$ in 1L bottles.

DAT = days after initial treatment; LSD = least significant difference

Table 4. Spectral normalized difference vegetative index measurements of Tifway bermudagrass based on iron + topramezone treatments. Results evaluated the treatment x run interaction for 2 experiments conducted during the months of August to September of 2018 in Auburn, AL.

| | NDVI[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 DAT | | 5 DAT | | 11 DAT | | 21 DAT | | 28 DAT | | 35 DAT | |
| Treatment[b] | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 |
| Non-treated Control | 0.726 | 0.742 | 0.706 ab | 0.745 | 0.693 a | 0.731 a | 0.700 a | 0.724 a | 0.708 a | 0.667 a | 0.712 | 0.680 a |
| Topramezone | 0.721 | 0.740 | 0.721 ab | 0.718 | 0.535 d | 0.526 d | 0.481 e | 0.427 f | 0.611 b | 0.467 f | 0.703 | 0.573 c |
| FeEDDHA<br>Topramezone | 0.728 | 0.739 | 0.727 a | 0.701 | 0.522 d | 0.512 d | 0.495 e | 0.465 e | 0.606 b | 0.506 e | 0.702 | 0.591 bc |
| FeDTPA<br>Topramezone | 0.731 | 0.744 | 0.708 ab | 0.711 | 0.527 d | 0.609 c | 0.547 cd | 0.610 c | 0.632 b | 0.585 c | 0.714 | 0.630 b |
| Iron Citrate<br>Urea<br>Topramezone | 0.742 | 0.737 | 0.719 ab | 0.693 | 0.595 b | 0.527 d | 0.643 b | 0.451 ef | 0.646 b | 0.490 ef | 0.713 | 0.593 bc |
| Ferrous Sulfate<br>Urea<br>Topramezone | 0.729 | 0.743 | 0.717 ab | 0.703 | 0.537 d | 0.554 d | 0.579 c | 0.536 d | 0.642 b | 0.546 d | 0.713 | 0.608 bc |
| Iron Oxide<br>Iron Sucrate<br>Ferrous Sulfate<br>Topramezone | 0.725 | 0.742 | 0.695 b | 0.696 | 0.535 d | 0.510 d | 0.527 de | 0.430 f | 0.631 b | 0.464 f | 0.712 | 0.553 c |
| Ferrous Sulfate<br>Topramezone | 0.715 | 0.737 | 0.710 ab | 0.710 | 0.570 c | 0.657 b | 0.628 b | 0.663 b | 0.651 b | 0.616 b | 0.695 | 0.646 ab |
| LSD | - | - | 0.01 | - | 0.02 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | - | 0.04 |
| *P* Value | NS | NS | 0.04 | NS | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS | <0.0001 |

[a] Means with the same letter in the same column are not statistically different based on Tukey's HSD ($\alpha = 0.05$)

[b] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

NDVI = normalized difference vegetation index; DAT = days after initial treatment; LSD = least significant difference

## Chapter 3.

## Micronutrient inclusion to reduce bermudagrass bleaching associated with topramezone

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

# Chapter 3. Micronutrient inclusion to reduce bermudagrass bleaching associated with topramezone

## Abstract

Topramezone offers excellent control of hard to control grassy weeds in bermudagrass; however, significant bleaching and necrosis symptoms follow application. Previous research addressing safening with FeDTPA and ferrous sulfate showed a reduction of bleaching and necrosis symptoms when applied in combination with topramezone. Research utilizing other micronutrients, which may offer similar results, is lacking. Two 4-week long greenhouse trials were conducted from April to May of 2019 (Auburn University Plant Science Research Center) to determine whether various micronutrients safened topramezone use on bermudagrass by reducing bleaching symptoms. Mixtures of topramezone (12.3 g ai $ha^{-1}$) and MSO (0.5% v $v^{-1}$) were applied in combination with five micronutrients at varying rates. Micronutrient sources were sodium borate, zinc sulfate, manganese sulfate, copper sulfate, and ferrous sulfate. Necrosis and bleaching symptoms were visually rated on a 0 to 100% scale. Reflectance (NDVI) readings were taken throughout the entirety of the trials. Treatments were arranged as a randomized complete block design with 4 replications. All data were analyzed using PROC GLM and Fisher's Protected LSD ($P<0.05$) was utilized for means separation. Results indicated that mixtures of topramezone + ferrous sulfate (all rates), zinc sulfate (all rates), copper sulfate (highest rates), and manganese sulfate (1 rate), reduced bleaching symptoms across all rating dates. Bermudagrass necrosis showed responses similar to that observed for bleaching. Mixtures of topramezone + ferrous sulfate and zinc sulfate produced the highest levels of safening across all treatments. As rates of Cu, Mn, and B increased they failed to lessen bleaching and necrosis, indicating potential antagonism with the herbicide. Future research should involve more thorough testing of these and other micronutrients for weed control efficacy and safening potential.

**Nomenclature:** Topramezone, [3-(4,5-dihydro-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone; bermudagrass, *Cynodon dactylon (L.) Pers.;* FeDTPA, chelated iron diethylenetriaminepentaacetic acid; MSO, methylated seed oil; NDVI, normalized difference vegetative index.

**Key words:** Topramezone, bermudagrass, micronutrient, ferrous sulfate, safener, zinc sulfate, bleaching, necrosis

## Introduction

Topramezone (Pylex®, BASF Corporation, Research Triangle Park, NC) has shown excellent potential for controlling various grassy weeds in cool- and warm-season grasses. Application of topramezone to susceptible turfgrass species such as bermudagrass (*Cynodon dactylon* L. Pers.) often results in an unsightly response characterized by an overall "bleaching" or whitening of the plant tissue, which affects the overall aesthetics of the applied area (Anonymous 2018). This bleaching effect appears first on newer plant tissue but is ultimately transient and short-lived (Brewer et al. 2017; Brosnan et al. 2011; Cox 2013; Cox et al. 2017; Elmore et al. 2011a, 2011b; Goddard et al. 2010; Grossmann and Ehrhardt 2007).

Multiple research experiments performed at Auburn from 2015-2018 (AP Boyd, personal communication) have shown that iron sulfate (Ferrous Sulfate Heptahydrate, Crown Technology Inc. Indianapolis, IN), and chelated iron - FeDTPA (Sprint® 330, BASF Corporation, Research Triangle Park, NC) when mixed with topramezone safened its use on bermudagrass by reducing overall bleaching and recovery time. Other micronutrients which also play a vital role in the growth of healthy turfgrass, including zinc, manganese, copper, and boron have not been previously tested for herbicide safening potential. It is common practice for turfgrass managers to mix herbicides, insecticides, fungicides, colorants or pigments, and fertilizers in the same spray tank to save time and labor costs. Some of these mixtures often include one or more of the previously listed micronutrients in the solution, and their effects on herbicide efficacy are often unknown.

When researching micronutrient usage in turfgrasses, it is evident that literature delving into the topic is lacking. There are arguably 17 elements that are essential for plant growth, which are divided into two groups: macronutrients and micronutrients (Salisbury and Ross

1992; Epstein and Bloom 2005; St. John et al. 2015). Macronutrients are those elements that are found in dry plant tissue in concentrations equal to or greater than 1000 mg $kg^{-1}$, while individual micronutrient concentrations are found at 100 mg $kg^{-1}$ or below (Salisbury and Ross 1992). When applied, this definition defines plant essential macronutrients as carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, calcium, magnesium, and sulfur, while micronutrients are defined as chlorine, iron, manganese, zinc, copper, boron, molybdenum, and nickel (St. John et al. 2015).

Evaluations of micronutrient use (other than Fe products) in turfgrass is relatively scant. The general perception is that native soils will typically provide sufficient micronutrients for turf growth. However, with constructed soils high in sand, such as putting greens and athletic fields, responses to micronutrient applications could be likely, and a few papers have evaluated this idea.

Annual application of boron (B) (0, 0.55, 1.1, 2.2 kg B $ha^{-1}$ yr) were split into monthly applications over two years to a creeping bentgrass putting green constructed on a native loamy sand never affected color, quality, clipping weight, thatch depth or shoot density (Guertal 2004). However, in a sand/peat greensmix, in the greenhouse, shoot density increased as B rate increased (weekly applied rates ranged from 0 – 3.0 mg $kg^{-1}$ B) (Guertal 2004). Work with copper (Cu) found that clipping weight and root mass were decreased by addition of Cu. In this greenhouse study, bentgrass growing in a sand-peat mix was never positively affected when Cu was added (Faust and Christians 2000).

When positive effects of micronutrient application to turf were observed it was often because the products were providing secondary benefits, rather than direct nutritional ones. This was observed with Mn applications and subsequent reductions in take-all patch

(*Gaeumannomyces graminis* (Sacc.) (Sacc.) Arx & D. Olivier var. *avenae* (E. M. Turner) Dennis.(Heckman et al. 2003). In other work, low application rates of Cu (2.44, 4.88, and 9.76 kg ha$^{-1}$) controlled moss (Boesch and Mitkowski 2005). However, as with previous work (Faust and Christians 2000) high concentrations of Cu in rootzones severely damaged turf (Boesch and Mitkowski 2005).

New turfgrass colorants or pigments, designed to protect plants from environmental stress, may also contain micronutrients. When pigments or paints that also contained Cu or Zn were evaluated, tissue Zn and Cu increased substantially. However, turfgrass health and quality was not improved by the application of any product (McCarty et al. 2014).

Application of Fe to turfgrass has long been utilized for the ability of that nutrient to green turf, without promoting excessive tissue growth (Carrow et al. 1988; Minner and Butler 1984; Yust et al. 1984). The most commonly applied Fe sources are Fe sulfate or Fe chelates (Cooper and Spokas 1991). Newer iron sources, such as Fe citrate, Fe glucoheptonate, and Fe humic acid materials have also been evaluated, although suitability as soil-applied sources varies widely with the product (Shaddox et al. 2018; 2019). In that work, soil application of Fe chelates was most effective, with other sources rendered ineffective for turfgrass response (Fe oxides, humates, sulfates, glucoheptonates, polysaccharides, citrates) due to rapid reductions in soil solubility (Shaddox et al. 2018; 2019).

In addition to color promotion, the use of Fe sulfate or Fe chelates as a safening material for herbicides has long been known. Researchers have found that iron can reduce symptoms of phytotoxicity associated with herbicide application on different turfgrass species (McCarty 1991; Price 1983; McCullough and Hart 2009; Johnson and Carrow 1995; Massey et al. 2006; Flessner et al. 2017). Research also showed that various rates of topramezone and chelated iron, when mixed, reduced bleaching of bermudagrass and did not reduce overall goosegrass control (Boyd et al. 2016a; 2016b).

In addition to safening work with Fe, herbicide interactions with Zn, Ca, Mg and Mn have also been studied. Rather than safening, inclusion of calcium, magnesium, or manganese antagonized the efficacy of 2,4-D on broadleaf plantain (*Plantago major* L.) and dandelion (*Taraxaxum officinale* F.H. Wigg*)* control when the nutrients formed weak acids following mixing with hard water sources (Patton et al. 2016). The inclusion of zinc had no effect on the efficacy of 2,4-D for these two weed species. These findings were similar to those observed by Roskamp et al. (2013), with horseweed (*Erigeron canadensis* (L.) Cronquist), kochia (*Kochia scoparia* (L.) Roth), redroot pigweed (*Amaranthus retroflexus* L.), and common lambsquarters (*Chenopodium album* L.). The research performed by Patton et al. (2016) aligns with previous findings by other researchers who tested hard water effects on aminopyralid, bentazon, dicamba, glufosinate, tembotrione, and glyphosate (Nalewaja and Matysiak 1993; Zollinger et al. 2010; Bernards et al. 2005; Roskamp et al. 2013; Chahal and Johnson 2012). Topramezone was not one of the herbicides tested during any of the research for antagonism or safening effects associated with hard water or micronutrients.

In other work, glyphosate weed control efficacy was reduced when mixed with zinc nutrient sources and unaffected when mixed with manganese nutrient sources on a variety of grassy and broadleaf weeds (Huber 2007). Reductions in weed control were reported when glyphosate and zinc were combined and applied to barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.), browntop millet (*Urochloa ramose* (L.) Nguyen), palmer amaranth (*Amaranthus palmeri* S. Wats.), johnsongrass (*Sorghum halepense* (L.) Pers.), ivyleaf morningglory (*Ipomeae hederacea* Jacq.), and redroot pigweed (Scroggs et al. 2009). The authors attributed the antagonism of glyphosate to higher concentrations of Zn ions in solution, which was similar to previous work focusing on magnesium and calcium ions in hard water performed by Mueller et al. (2006). Similarly, control of velvetleaf (*Abutilon theophrasti* Medik.), giant foxtail (*Setaria faberi* Herm.), and common lambsquarters were reduced when manganese was applied with glyphosate, indicating antagonism (Bernards et al. 2005). Conversely to all the previous work, combinations of bromoxynil + MCPA and 2,4-D + MCPA showed no efficacy loss of broadleaf weed control when mixed with chelated combination products that included molybdenum, iron, zinc, copper, magnesium, cobalt, manganese, and nitrogen (Meybodi et al. 2011). Also, application of boron in combination with haloxyfop-methyl, sethoxydim, clethodim, and fluazifop-p-butil did not antagonize weed control or increase crop injury in sunflower (*Helianthus annuus* (L.))(Brighenti and Castro 2008).

Thus, previous research tested a multitude of micronutrient and herbicide combinations for effects of synergism and antagonism, but micronutrients and topramezone combinations have not been previously tested. Since previous research has shown that various iron sources safened topramezone when mixed and applied to bermudagrass, and no other research exists testing other micronutrients for safening potential or antagonism, the objective of this study was to evaluate different rates of micronutrients in combination with topramezone to determine whether a reduction of bleaching or necrosis was observed.

## Materials and Methods

Two greenhouse studies (2018-2019) were conducted at the Auburn University Plant Science Research Center in Auburn, AL (32°34'39.26"N, 85°29'55.32"W). The two studies, designated as Run 1 and Run 2, were initiated on April 3rd and April 17th of 2019 and concluded on May 15th and 29th of 2019.

'Tifway' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy] plugs measuring 10.2 cm in diameter, and 12.7 cm in depth were collected from the Auburn University Turfgrass Research Unit and used for all studies. The soil type of the area was a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) with a pH of 6.1. Bermudagrass plugs were collected and placed in a greenhouse maintained at 31.7°C with supplemental light sources (Mebulbs, High Intensity Discharge Metal Halide 1500 W, Fargo, ND) programmed for a long day light cycle of 15 hours. The bermudagrass was clipped at 2.5 cm as needed while acclimating to greenhouse conditions. Clippings prior to the initiation of the study were removed and discarded. Pots were clipped one day prior to foliar application of treatments and clipped every 7 days after application to maintain the 2.5 cm height. Clippings were collected every 7 days and dried for 48 h at 60°C in a drying oven prior to weighing the dry matter. All pots were observed to have dense stands at the initiation of each trial. Pots were watered every other day to maintain adequate moisture levels near field capacity.

Foliar applied treatments were made using a handheld four nozzle boom fitted with TeeJet TP8002VS (TeeJet Spraying Systems, Roswell, GA) nozzles on 22.9 cm spacing and pressurized with a $CO_2$ backpack cylinder calibrated to deliver 280 L $ha^{-1}$ at 172 kPa. All plants were moved outside for applications with all four replications sprayed concurrently. Topramezone was applied in combination with 5 micronutrients, each applied at 3 rates (Table 1). Micronutrient rate selection was based on previous research (Boyd et al. 2016a, 2016b) and suggested rates in the literature (Marschner 1995; Epstein 1972; Salisbury and Ross 1992). All treatments included methylated seed oil (MSO, Alligare, Opelika, AL) at 0.5% v $v^{-1}$. These treatments were compared to topramezone (12.3 g ai $ha^{-1}$) applied alone, as well as a non-treated check. All treatments were applied on the initial start date, and no additional applications of herbicide, fertilizer, or fungicide were made during the course of the trials.

Visually estimated bermudagrass symptoms of bleaching and necrosis were assessed at 3, 7, 14, 21, 28, 35, and 42 days after initial treatment (DAT). Bleaching was measured on a 0-100% scale where 0% was defined as no observed bleaching and 100% as complete whitening of plant tissue. Necrosis was measured on a 0-100% scale where 0% was defined as no observed tissue death and 100% was total death and browning of the plant tissue.

Spectral NDVI analysis was measured using a handheld multispectral radiometer (Spectrum Technologies, Inc., Model TCM 500 Turf Color Meter). Readings were taken by placing the unit on the surface of each plug for capture. Four readings were taken per pot and the average NDVI was recorded. Reflectance measurements were collected at 0, 3, 7, 14, 21, 28, 35, and 42 DAT during the greenhouse trials. The NDVI measurements were calculated using the following formula:

$$\text{NDVI}= \frac{R_{780} - R_{670}}{R_{780} + R_{670}} \qquad [1]$$

where $R_{780}$ and $R_{670}$ are designated as the measured reflectance of near-infrared radiation (780 nm) and visible red radiation (670 nm) (Bremer et al. 2011; Trenholm et al. 1999).

The study was conducted as a factorial arrangement of 5 micronutrients, each at 3 rates, plus two controls (topramezone and a non-treated control), arranged in a randomized complete block design with 4 replications of each combination. Data were analyzed within PROC GLM using Fisher's Protected LSD ($P < 0.05$) in SAS 9.4 (SAS Institute, Cary, NC, 27513).

Correlations of data were run using PROC CORR in SAS 9.4. Graphs were plotted using SigmaPlot 13.0 (Systat Software Inc.).

## Results and Discussion

**Bleaching.** Interactions of treatment-by-run-by-DAT (P=0.9996), treatment-by-run (P = 0.896) and run-by-DAT (0.0557) were not significant. The interaction of treatment-by-DAT (P < 0.0001) was significant. Correlations comparing bleaching with NDVI were strongly negative (-0.85704) and statistically significant (P < 0.0001) (Table S3). All other variables were weakly correlated and will not be discussed (Table S3). Data were pooled across both runs and are presented in Figure 1.

Bleaching symptoms occurred as early as 7 DAT and dissipated by 21 DAT for most treatments (Figure 1). At 7 DAT, differences in bleaching due to the inclusion of micronutrients was only observed in treatments that contained Fe or Cu (0.78 kg ha$^{-1}$ rate only)(Figure 1). By 14 DAT, any rate of Cu, Zn or Fe reduced bermudagrass bleaching beyond that observed in the topramezone-only treatment. Only the highest rate of Mn reduced bermudagrass bleaching, when compared to bermudagrass receiving topramezone-alone. Bermudagrass to which B had been applied had mixed results: reductions in bleaching at the lowest and middle B rates, but no effect at the highest.

Treatments which produced greatest reductions in bleaching of bermudagrass were zinc sulfate (highest rate), manganese sulfate (highest rate), copper sulfate (two highest rates), and iron sulfate (all rates). Previous research has shown that additions of zinc, manganese, and copper have had antagonistic effects when combined with glyphosate due to the formation of complexes with the herbicide, rendering the herbicide less effective (Bernards et al. 2005;

Huber 2007; Scroggs et al. 2009). These data confirm previous research findings, which determined that iron sulfate safened topramezone on bermudagrass (Boyd et al. 2016a). It also indicates that safening occurred over a range of Fe rates. These data also corroborate previous findings by other researchers who used different herbicides with chelated iron to reduce turfgrass injury (McCullough and Hart 2009; Johnson and Carrow 1995; Massey et al. 2006; Flessner et al. 2017). Since the rates listed for manganese sulfate, copper sulfate, and zinc sulfate are the highest rates tested in this trial, antagonism of the herbicide and its efficacy could explain the observed reduction in bleaching. This work did not address the effects of this safening on any reduction in weed control efficiency, as effects on grassy weeds were not an objective of this work.

Previous work that focused on iron, does indicate that the addition of Fe safens topramezone, but does not affect grassy weed control. Results showed that when topramezone and FeDTPA were combined at varying rates and applied to goosegrass, complete control was achieved with no apparent loss of efficacy (Boyd et al. 2016). The addition of zinc sulfate, manganese sulfate, and copper sulfate offers other micronutrient options for future research on topramezone safening and weed control efficacy.

**Necrosis.** The interactions of treatment-by-run-by-DAT ($P = 0.9786$), run-by-DAT ($P = 0.9652$), and treatment-by-run ($P = 0.8873$) were not significant, while the interaction of treatment-by-DAT ($P < 0.0001$) was significant. Correlations comparing necrosis with bleaching were found to be significant (Table S3). Data were pooled across runs and results are presented in Figure 2.

Necrosis was first noted at 21 DAT and had mostly disappeared by 35 DAT (Figure 2). If following the guidelines of acceptability for necrosis being less than 20% damage, 6 out of

16 treatments had acceptable bermudagrass at 21 DAT, and 11 out of 16 were acceptable at 28 DAT. At 21 DAT, all treatments resulted in reduced levels of necrosis in the bermudagrass, when compared to that to which topramezone had been applied. The only exception to this was in the Mn treatments, where bermudagrass to which the two lower rates of Mn had been applied had necrosis equal to that found in the bermudagrass treated with topramezone-only. The highest rate of Mn did significantly reduce necrosis.

Similar results were observed at 28 DAT, with every rate of B, Zn, Cu and Fe reducing necrosis beyond that observed in the topramezone-only control. As at 21 DAT, inclusion of Mn (now at the two highest rates) reduced necrosis, while Mn inclusion at the lowest rate did not. By 35 DAT all bermudagrass was equal in damage, and that damage was largely at zero (Figure 2). The only exception to this was, once again, bermudagrass to which Mn was applied, with necrosis still visible in some bermudagrass (approximately 20% at the lowest Mn rate).

Treatments which produced the greatest reductions in bermudagrass necrosis when combined with topramezone included sodium borate (two lowest rates), zinc sulfate (all rates), manganese sulfate (highest rate), copper sulfate (middle rate), and iron sulfate (all rates). These findings were similar to those observed with bleaching. Previous research involving B showed that the inclusion of sodium borate in a mixture had no effect on herbicide efficacy (Brighenti and Castro 2008). All rates of sodium borate reduced overall necrosis in our study, but as rates increased, levels of necrosis also increased. Additional work showed zinc, copper, and manganese all had antagonistic effects on herbicide efficacy when mixed with other herbicides (Bernards et al. 2005; Huber 2007; Scroggs et al. 2009). Weed control was not tested in this study, but observed reductions in bermudagrass necrosis for zinc sulfate, copper sulfate, and manganese sulfate could be related to antagonism of the nutrient with topramezone. Further research is needed to determine whether these mixtures reduce herbicide efficacy.

These data confirm previous research findings which determined that iron sulfate safened topramezone on bermudagrass (Boyd et al. 2016a). It also shows reductions in necrosis at a range of Fe rates. Previous work showed that topramezone mixed with chelated iron at multiple rates was not antagonistic and did not reduce herbicide efficacy for goosegrass control (Boyd et al. 2016b). These data also corroborate previous findings by other researchers who used different herbicides with chelated iron to reduce turfgrass injury (McCullough and Hart 2009; Johnson and Carrow 1995; Massey et al. 2006; Flessner et al. 2017).

**Clipping Yield.** The treatment-by-run interaction, main effect of run and treatment were all non-significant across both trials in 2019. Since these data were found to be not significant, the corresponding data will not be reported.

**Normalized Difference Vegetation Index.** The interactions of treatment-by-run-by-DAT ($P = 0.835$), treatment-by-run ($P = 0.923$) and run-by-DAT ($P = 0.9267$) were not significant, while the interaction of treatment-by-DAT ($P < 0.0001$) was significant. Correlation data comparing NDVI with bleaching found a statistically significant ($P < 0.0001$) strong negative linear pattern with a r value of -0.85704 (Table S3). This relationship suggests that when bleaching levels increase, NDVI readings decrease or vice versa. Data were pooled over the runs and presented in Figure 3. Since reflectance data is best compared to untreated turf, which would retain color, that treatment is also included in Figure 3.

At 7 DAT, any bermudagrass receiving topramezone with or without micronutrients had significantly lower NDVI readings than measured in the non-treated control (Figure 3). There were few differences due to nutrient, within the topramezone treatments. At 14 DAT the addition of Mn (highest rate), Zn (highest rate), Cu (two highest rates) and Fe (all rates) increased bermudagrass reflectance as compared to the topramezone-only treatment, but reflectance was still well below that measured in the non-treated control. This general trend continued for 21 DAT, after which reflectance was equal in all treatments (28 DAT).

It is important to discuss what the NDVI device is actually measuring in order to comprehend the given values. A spectral radiometer measures spectral and radiative differences correlated to the red and near infrared (NIR) wavelengths, which produces a number from -1 to +1 (Bremer et al. 2011). When an area of turfgrass is measured, values generally range from 0.2 - 0.5 which includes bare ground, turf dormancy, moisture content, and injury or necrosis, while values of 0.5 – 0.9 demonstrate turfgrass with differing levels of plant health and chlorophyll content (Bell et al. 2004; Bremer et al. 2011; Stiegler et al. 2005; Goodin and Henebry 1998). High chlorophyll content in the plant tissue translates to a lower reflectance value of the red wavelength and a higher reflectance value of NIR, which results in a higher reflectance value and the appearance of green tissue to the human eye (Gausman 1977). Alternatively, a low chlorophyll content will reflect higher levels of the red-light spectrum and lower levels of NIR. This results in lower reflectance values and a brown or yellowish appearance to the observer (Bremer et al. 2011; Karcher and Richardson 2003, 2005; Trenholm et al. 1999).

Previous work involving reflectance values has shown a lot of variation from the subjective measures of color and quality ratings. Large variations in NDVI values have been reported in studies where turfgrass color is all equal, but due to factors such as species, cultivar, moisture level, fertility level, and shade exposure, reflectance values were skewed (Bremer et al. 2011; Brosnan et al. 2005; Gausman 1977; Goodin and Henebry 1998; Penuelas et al. 1993).

In previous work at Auburn University, a handheld multispectral radiometer (Holland Scientific, Model ACS-430 Crop Circle) was mounted to a golf trolley above the surface and pushed over field studies while collecting data (Boyd et al. 2016a). The data recorded from these studies followed closely with the visual symptoms of bleaching and necrosis observed during the trials. Correlation values comparing NDVI with bleaching (r = -0.38214; moderate) or necrosis (r = -0.79435; strong) both resulted in negative linear relationships and were statistically significant ($P < 0.0001$). In our study, a handheld multispectral radiometer was placed on the surface of the bermudagrass plug, which in turn compressed the canopy while readings were being measured. This compression of canopy structure resulted in varied results that potentially skewed the data from that of the visual ratings of bleaching and necrosis. Correlation values comparing NDVI with bleaching found a statistically significant ($P < 0.0001$) strong negative linear pattern with a r value of -0.85704, but a very weak positive correlation when compared to necrosis (r = 0.08153). Although all bermudagrass was measured with the same device in this study, reflectance readings which are normally very consistent were variable. These findings are important to mention, since other researchers have not reported similar issues with canopy compression.

Differences among the treatments including manganese sulfate, copper sulfate, and zinc sulfate showed that when mixed with topramezone, higher NDVI readings resulted when compared to that of topramezone applied alone. As stated before, these micronutrients have shown antagonism when mixed with other herbicides. Antagonism of topramezone could be one reason for the higher NDVI readings, while potential safening at the listed rates is the other. More research is needed to determine whether these micronutrient combinations offer safening at the target site when mixed with topramezone.

## Acknowledgments

The authors would like to thank Jane Farr and her team at the Auburn Plant Science Research Center for providing the greenhouse space used during the duration of the experiments. Thanks also go to David Lawrence and his crew at the Auburn Sports Surface Field Laboratory for providing the bermudagrass plugs used during the experiment and to Kathie Kalmowitz and BASF for providing the topramezone used throughout all of the trials. No conflicts of interest have been declared.

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Table 1. List of treatments[a] and rates used for topramezone + micronutrient greenhouse trials on bermudagrass in Auburn, AL in 2019.

| Active Ingredient | Trade Name | Manufacturer | Topramezone rate | Micronutrient rate |
|---|---|---|---|---|
| | | | g a.i. ha$^{-1}$ | kg nutrient ha$^{-1}$ |
| Topramezone | Pylex® | BASF<br>Research Triangle Park, NC | 12.3 | --- |
| Topramezone<br>Sodium borate | Sodium borate ($BNa_3O_3$) | VWR<br>Radnor, PA | 12.3 | 0.09; 0.17; 0.34 |
| Topramezone<br>Zinc sulfate | Zinc sulfate ($ZnSO_4$) | VWR<br>Radnor, PA | 12.3 | 0.69; 1.39; 2.08 |
| Topramezone<br>Manganese Sulfate | Manganese sulfate ($MnSO_4$) | VWR<br>Radnor, PA | 12.3 | 0.99; 1.98; 2.98 |
| Topramezone<br>Copper sulfate | Copper sulfate ($CuSO_4$) | VWR<br>Radnor, PA | 12.3 | 0.39; 0.78; 1.55 |
| Topramezone<br>Ferrous sulfate | Ferrous sulfate ($FeSO_4$) | Crown Technology Inc.<br>Indianapolis, IN | 12.3 | 0.61; 1.22; 2.44 |

[a] All treatments were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v v$^{-1}$ in 1L bottles.

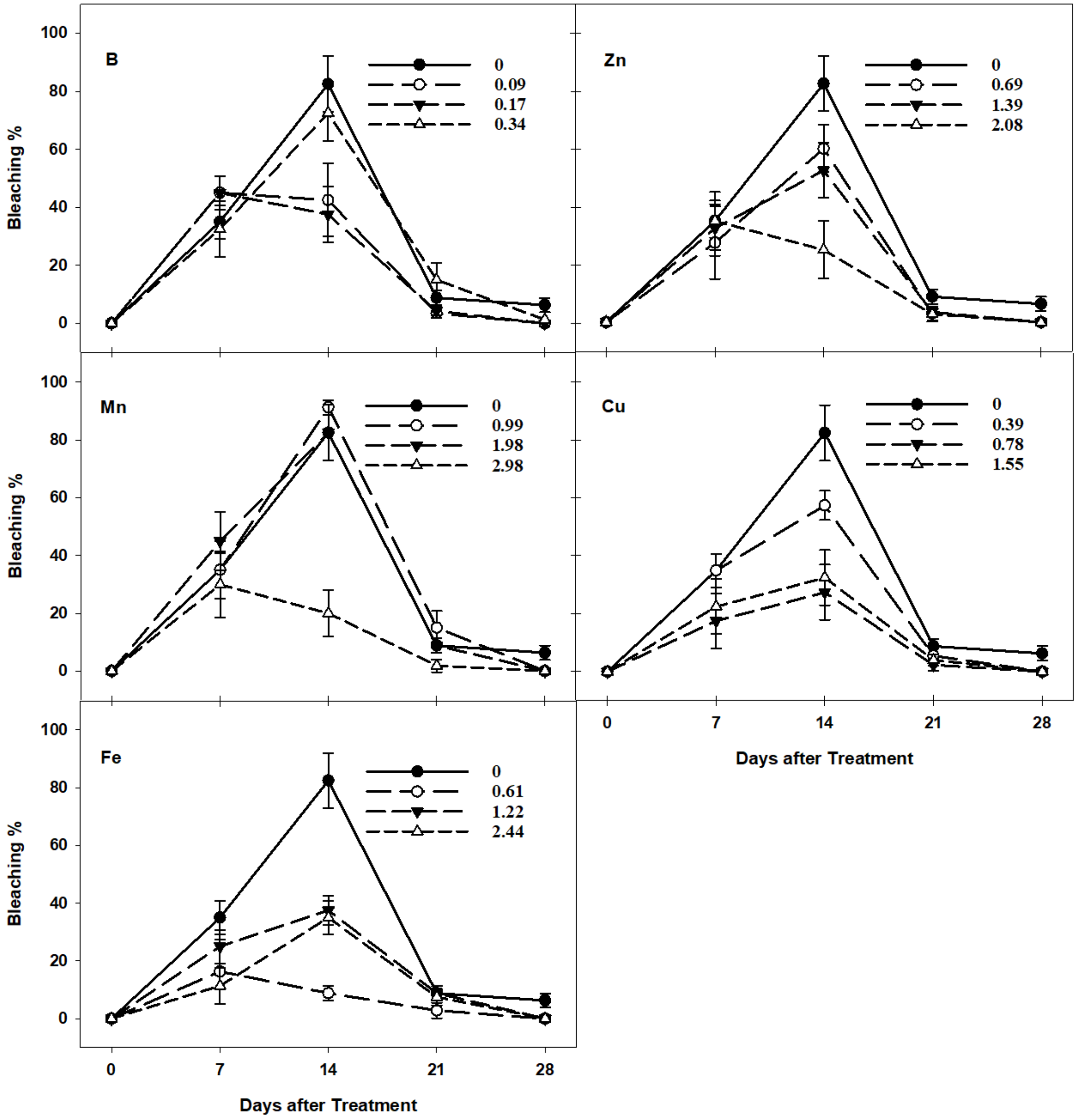

Figure 1. Bermudagrass bleaching percentage following treatments of topramezone alone and in combination with micronutrients at 0, 7, 14, 21, and 28 days after treatment. All treatments included topramezone at 12.3 g a.i. ha$^{-1}$ and methylated seed oil at 0.5% v v$^{-1}$. Micronutrient rates are listed in kg nutrient ha$^{-1}$ from the products sodium borate (B), zinc sulfate (Zn), manganese sulfate (Mn), copper sulfate (Cu), and iron sulfate (Fe). Greenhouse research conducted from April-May of 2019 in Auburn, Alabama. Results evaluate the main effect of treatment and are pooled over the two trials. Error bars indicate standard deviation of the individual means, *n* = 8.

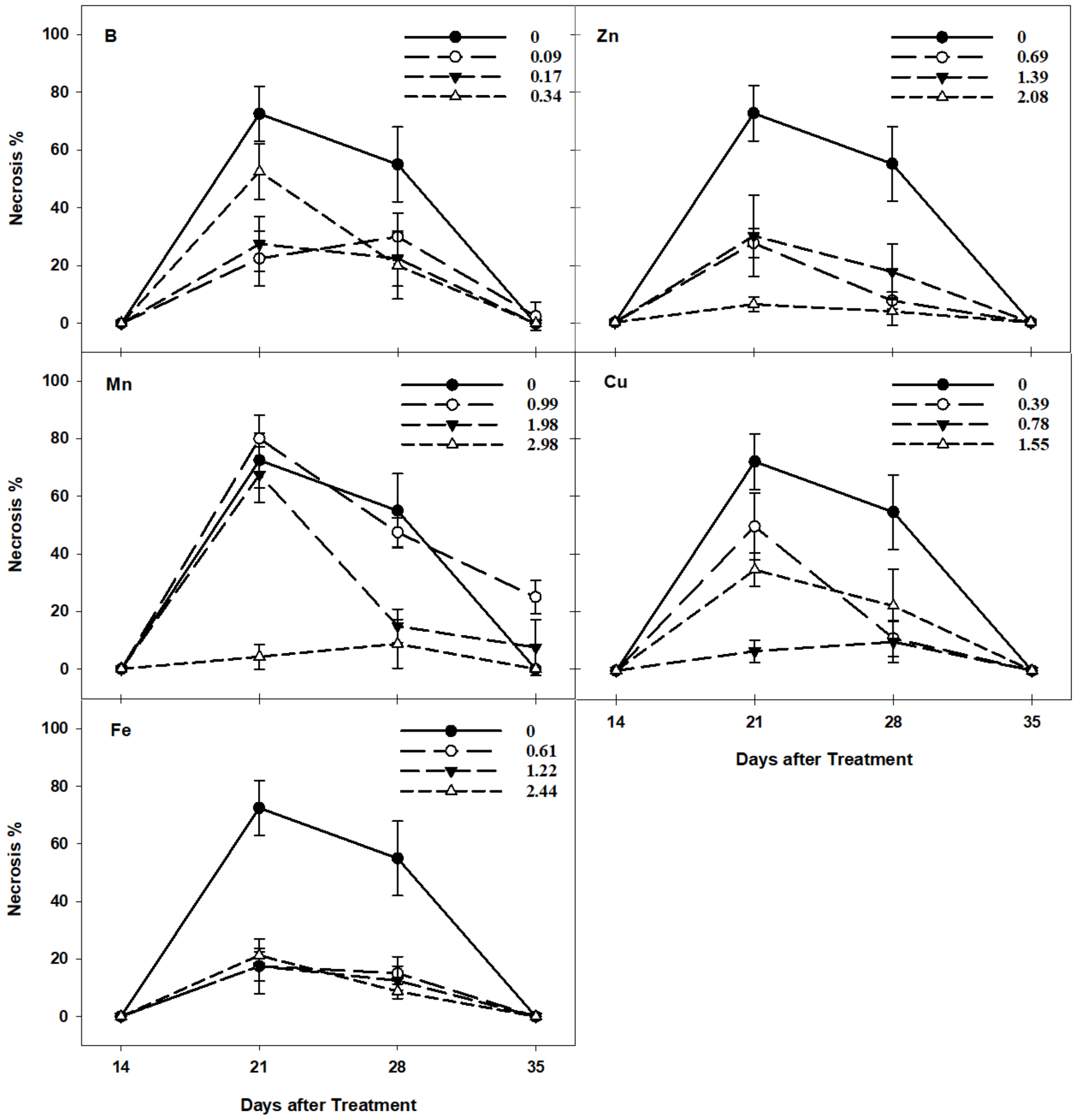

Figure 2. Bermudagrass necrosis percentage following treatments of topramezone alone and in combination with micronutrients at 14, 21, 28, and 35 days after treatment. All treatments included topramezone at 12.3 g a.i. $ha^{-1}$ and methylated seed oil at 0.5% v $v^{-1}$. Micronutrient rates are listed in kg nutrient $ha^{-1}$ from the products sodium borate (B), zinc sulfate (Zn), manganese sulfate (Mn), copper sulfate (Cu), and iron sulfate (Fe). Greenhouse research conducted from April-May of 2019 in Auburn, Alabama. Data were pooled over the two trials and evaluate the main effect of treatment over time. Error bars indicate standard deviation of the individual means, *n* = 8.

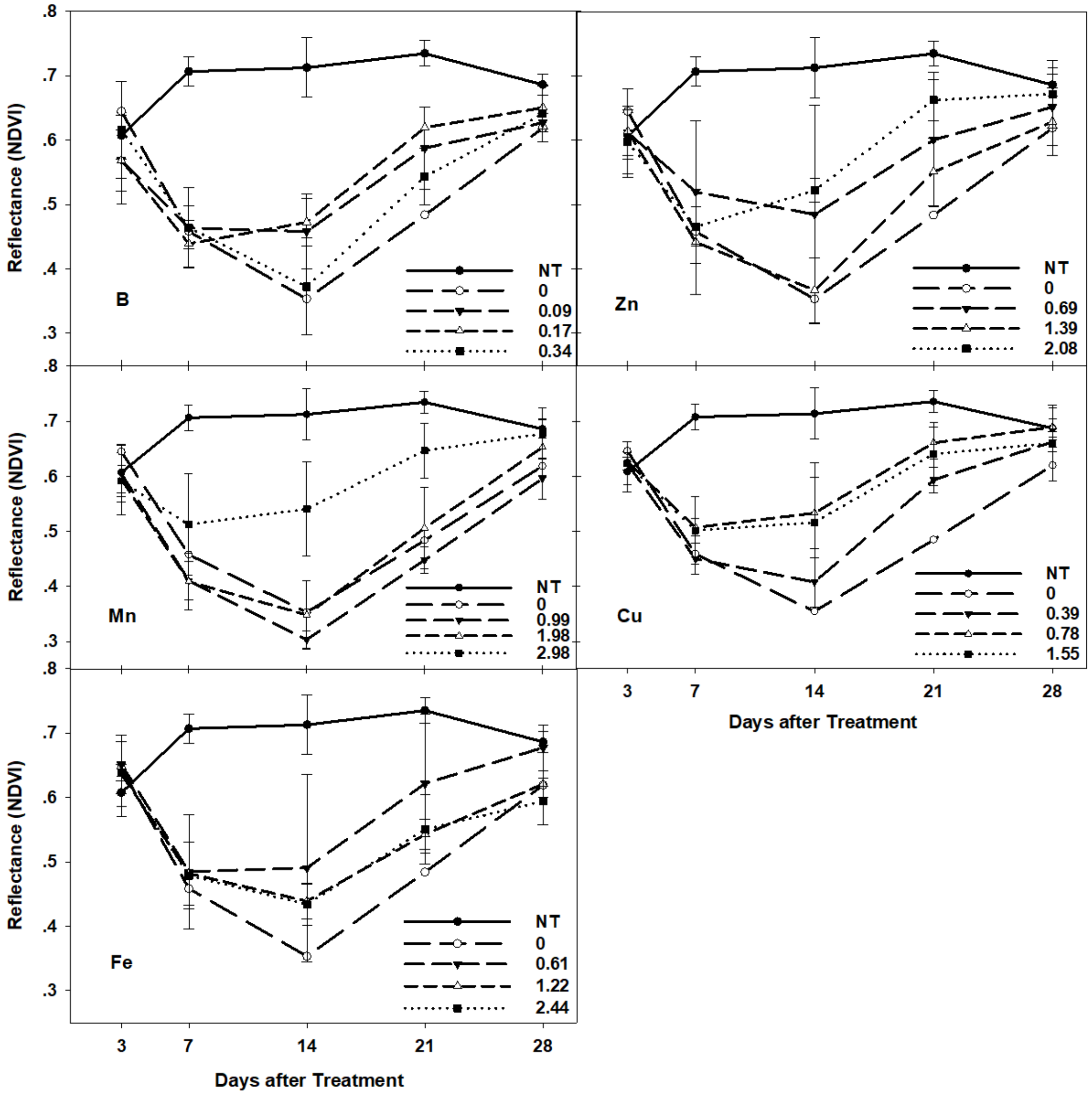

Figure 3. Bermudagrass reflectance (NDVI) values following treatments of topramezone alone and in combination with micronutrients at 3, 7, 14, 21, and 28 days after treatment. All treatments included topramezone at 12.3 g a.i. ha$^{-1}$ and methylated seed oil at 0.5% v v$^{-1}$. Micronutrient rates are listed in kg nutrient ha$^{-1}$ from the products sodium borate (B), zinc sulfate (Zn), manganese sulfate (Mn), copper sulfate (Cu), and iron sulfate (Fe). A non-treated (NT) check was also included. Greenhouse research conducted from April-May of 2019 in Auburn, Alabama. Data were pooled over the two trials and evaluate the main effect of treatment over time. Error bars indicate standard deviation of the individual means, *n* = 8.

# Chapter 4.

## Topramezone, Iron and Zinc Combinations on Hybrid Bermudagrass Physiological Responses.

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

# Chapter 4. Topramezone, Iron and Zinc Combinations on Hybrid Bermudagrass Physiological Responses

## Abstract

Topramezone is an excellent herbicide for difficult to control weeds, but resultant bleaching and necrosis of the desired turfgrass is not acceptable in turfgrass settings. It is known that chelated iron, iron sulfate, or zinc sulfate safens topramezone when mixed and applied to bermudagrass. Although safening occurs, the exact mechanism for this is not known. Thus, the objective of this project was to examine temperature/daylength and selected micronutrient effects on topramezone safening and physiological indicators. In the growth chamber, bermudagrass was subjected to summer (31.9/20.8°C day/night, 14 daylight hours) and fall (23.9/12.2°C day/night, 11.5 daylight hours) conditions commonly found in the southeast US. Within each season there were factorial combinations of topramezone and three nutrient sources: chelated iron/N, iron sulfate, and zinc sulfate. Tissue bleaching, total canopy photosynthetic efficiency, clipping yield, and pigment concentrations of chlorophyll *a*, chlorophyll *b*, and total carotenoids were measured at 0, 7, 14, 21, and 28 days after treatment. Applications of topramezone reduced total canopy photosynthetic efficiency, regardless of the temperature/daylength regime. Greatest reductions in pigment concentration occurred in chlorophyll *a* and total carotenoids, while effects on chlorophyll *b* were largely unchanged. Application of iron (but without N) in combination with topramezone to bermudagrass helps to stabilize tissue bleaching by increasing chlorophyll production, while the plant functions to metabolize the herbicide.

**Nomenclature:** Topramezone, [3-(4,5-dihydro-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone; bermudagrass, *Cynodon dactylon (L.) Pers.;* FeDTPA, chelated iron diethylenetriaminepentaacetic acid;

**Key words:** Topramezone, bermudagrass, pigment concentration, iron sulfate, safener, zinc sulfate, bleaching

## Introduction

Topramezone (Pylex®, BASF Corporation, Research Triangle Park, NC) is a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor which affects carotenoid biosynthesis by restricting the synthesis of HPPD, in turn preventing the production of plastoquinone, phytoene desaturase, phytoene, and phytofluene, all key components in carotenoid biosynthesis (Bollman et al. 2008; Brosnan et al. 2011; Buchanan et al. 2000; Grossman and Ehrhardt 2007; Mitchell et al. 2001; Secor 1994). Reduction in reactions involving phytoene desaturase is associated with application of HPPD inhibitors and linked to an overall decrease in carotenoid generation (Grossman and Ehrhardt 2007; Brosnan et al. 2011). Additionally, a reduction in tocopherols, also due to HPPD application, results in unstable oxygen singlets previously regulated by carotenoids, freely degrading chlorophyll and photosynthetic membranes within the newly formed shoot tissue (Grossman and Ehrhardt 2007). This leads to whitening or bleaching of the newest growth on the plant (Anonymous 2018).

Unfortunately, bleaching of desirable turfgrass species also occurs, resulting in undesirable phytotoxicity and injury. Many treatments have been explored to lessen bleaching, including nutrients, cultural practices, herbicides, or chemical safeners (Boyd et al. 2016, 2017, 2018, 2020; Brewer et al. 2017; Elmore et al. 2011a, 2011b; Kerr et al. 2019). Even though bleaching effects tend to be transient, typically lasting 7-14 days, the striking symptoms of injury are difficult to overlook. Attempts to safen topramezone usage on turfgrass species such as bentgrass (*Agrostis stolonifera* L.) and bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) have been researched (Boyd et al. 2016, 2018; Cox 2013; Cox et al. 2017; Elmore et al. 2015a, 2015b; Goncalves 2019; Kerr et al. 2019). Previous work has shown the nutrient sources iron chelate (FeDTPA), iron sulfate, and zinc sulfate safened topramezone applied to bermudagrass (Boyd et al. 2016, 2018, 2020). However, the specific safening mechanism is not yet known. Additionally, season affected bleaching symptoms, with bleaching occurring earlier in warmer conditions and delayed in cooler conditions. (Boyd et al. 2016, 2020).

The application of Fe (Fe chelate, specifically) has sometimes been shown to safen other herbicides (Flessner et al. 2017; Johnson and Carrow, 1995; McCullough and Hart, 2009), but results are mixed. In some cases, the application of Fe merely masked symptoms, rather than actually safening (McDonald et al. 2006). In other work, Fe applications did reduce bermudagrass injury, but it also reduced herbicide effectiveness (Massey et al. 2006). It is known that application of foliar iron increases plant chlorophyll production, creating green color without leaf growth (Stiegler et al. 2003), which provides the masking effect (McDonald et al. 2006). However, the exact mechanism by which Fe provides any safening (beyond masking) is not elucidated, and specifically not for topramezone.

Evaluations of Zn as a safener in turfgrass systems found negative or no results, with the addition of that micronutrient to various herbicides (glyphosate and 2,4-D, primarily) resulting in reduced herbicide efficacy (Huber, 2007; Scroggs et al. 2009), or no effect (Patton et al. 2016; Meybodi et al. 2011). None of that work examined combinations of Zn and topramezone. While previous work (Boyd et al. 2020) demonstrated that Zn did provide topramezone safening, the mechanism for that is not yet understood.

Herbicide research which studied pigment concentrations in bermudagrass leaf tissue has been evaluated previously. Application of three HPPD inhibiting herbicides (topramezone, tembotrione, and mesotrione) all reduced total pigment concentrations in “Riviera” bermudagrass following application, with greatest reductions from applications of topramezone and tembotrione (Brosnan et al. 2011). At 21 days after treatment (DAT), approximately 7 days after peak bleaching symptoms, increased levels of zeaxanthin and antheraxanthin were observed. They concluded that the increase of these photoprotective carotenoid pigments was a mechanism which allowed the recovery of bermudagrass following HPPD inhibitor application (Brosnan et al. 2011). Additional work with mesotrione reported that bermudagrass pigment concentrations decreased after application, followed by rapid increase in chlorophyll and carotenoid concentrations as plants recovered (Kopsell et al. 2010). Additional mesotrione research reported that chlorophyll *a*, chlorophyll *b*, beta-carotene, lutein, and violaxanthin all decreased following application, while levels of phytoene and zeaxanthin increased (McCurdy et al. 2008). So, there is established evidence that application of HPPD herbicides affects various plant pigments. However, none of the studies included safening treatments.

Research studying the physiological effects on pigment concentration and overall photosynthesis output following the application of topramezone to bermudagrass, especially when safeners are included, is limited. Previously research has identified Fe and Zn as safeners for topramezone, but we do not yet know the mechanism for that safening. Insight into how the addition of zinc or iron to topramezone changes pigment concentrations or alters photosynthesis would help us better understand the mechanism of this safening. Thus, the objective of this study was to evaluate photosynthesis response and pigment changes to hybrid bermudagrass subjected to two temperature/daylength regimes (seasons), following application of topramezone with or without FeDTPA/N, iron sulfate, and zinc sulfate.

## Materials and Methods

**Plant Culture.** 'Tifway' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy] plugs measuring 10.2 cm in diameter and 15.3 cm in depth were collected from the Auburn Turfgrass Research Unit (32°34'N, 85°29'W) and used for all studies. Soil type in the bermudagrass area used for all runs was a Marvyn sandy loam with a pH of 5.9 (fine-loamy, kaolinitic, thermic Typic Kanhapludult). Harvested plugs were placed in pots that were 12.7 cm deep and 10.2 cm by 10.2 cm at the top and tapered to 8.3cm by 8.3 cm at the bottom (Dillen Products, Twinsburg, OH). Harvested plugs were tapered to match the interior of each pot. Any additional soil used to fill voids in the pot was taken from the removed soil created during the tapering process. Each set of bermudagrass treatments was replicated 3 times. The four-year-old bermudagrass area from where the plugs were collected was maintained at fairway height (2.5 cm) and received a monthly fertilizer regimen of 24.4 kg N $ha^{-1}$. Bermudagrass plugs used in the studies were collected in late February and March and placed directly in the growth chamber for an acclimatization period of 7 days. On these collection dates, prior to acclimatization, the bermudagrass had broken dormancy and was generally green. The bermudagrass was hand clipped one day prior to treatment applications, and clipped every 7 days after application to maintain a height of 3.8 cm. All samples were observed to have dense stands at the initiation of each trial. Plants were watered every other day to maintain adequate moisture levels near field capacity. Each experiment lasted for 28 days and was repeated in time.

**Growth Chamber Specifics.** Growth chamber trials were conducted in growth chambers (Conviron Adaptis, Conviron, Winnipeg, Canada) from March 2020 until May of 2020. Treatments were nutrient sources and seasonal conditions simulated by daylength/temperature. Daylength/temperature treatments were based on 30-year averages in Auburn, AL (usclimatedata.com), and simulated seasonality of summer and fall in the northern hemisphere. Summer temperature settings were set at 31.9°C for 14 daytime hours, and 20.8°C during night time hours to simulate conditions found in Auburn, AL in July/August. Fall temperature settings were set at 23.9°C for 11.5 daytime hours and 12.2°C during night time hours to simulate conditions found in Auburn, AL in October/November. Hereafter for the remainder of this paper the daylength/temperature regimes will be referred to as 'summer' and 'fall' seasons. Specific application dates and run names are listed in Table 1. Relative humidity was set at 50% in the growth chambers to limit disease pressure during the trials. Pots were rotated every 7 days between two shelves within each growth chamber to reduce any chamber effects on the studies.

**Nutrient Treatments.** Foliar nutrient treatments were applied using a handheld, four-nozzle boom fitted with TeeJet TP8002VS (TeeJet Spraying Systems, Roswell, GA) nozzles on 22.9 cm spacing and pressurized with a $CO_2$ backpack cylinder calibrated to deliver 280 L $ha^{-1}$ of spray solution at 172 kPa in accordance with suggested application rates. Topramezone was applied in combination with chelated iron- FeDTPA/N, iron sulfate, and zinc sulfate (Table 2). The three nutrient sources were also applied alone, without inclusion of topramezone. All treatments which included topramezone were mixed with methylated seed oil (MSO, Alligare, Opelika, AL) at 0.5% v $v^{-1}$. These treatments were compared to topramezone (12.3 g ai $ha^{-1}$) applied alone, as well as a non-treated check. With a non-treated control and a topramezone-only control, there were a total of 8 nutrient/topramezone treatments, each replicated three times in the specific growth chamber for a seasonal treatment. This entire experiment was conducted two times, with the season treatment switched from one growth chamber to another each time. All treatments were applied on the indicated start date, with no additional applications applied over the duration of the trial (Table 1).

**Data Collection.** Visually estimated bermudagrass symptoms of bleaching were assessed at 0, 7, 14, 21, and 28 DAT where 0% was defined as no observed bleaching and 100% as complete whitening of plant tissue.

Bermudagrass was hand-clipped to a height of 3.8 cm at 0, 7, 14, 21, and 28 DAT, and fresh weight was measured. Each clipping event took place right after the scheduled $CO_2$ measurement for that day. After weighing, clipping biomass was immediately frozen in liquid nitrogen and placed on ice to allow for transport to storage at -80°C. Subsequent pigment concentration processing and analysis took place at the completion of all trials.

**$CO_2$ Sequence and Canopy Photosynthesis Measurements**. Whole pot canopy photosynthesis measurements were collected using a closed system, modular transparent custom-built chamber (Soba et al. 2020) (Figure 1). The chamber consisted of two sections including a base and top module. The base module acted as a resting platform for the plant material, as well as a means to seal the chamber. The top module functioned as a ceiling and a carrier for all sensors and tube fittings (Figure 1). A temperature sensor (LI-1000-8, LI-COR Biosciences, Lincoln, NE, USA), photosynthetically active radiation (PAR) sensor (LI-190, LI-COR Bioscience, Lincoln, NE, USA), and 5 m of polyetra-fluoroethylene (PTFE) tubing were connected to the LI-8100 (LI-COR Bioscience, Lincoln, NE, USA) which served as a $CO_2$ analyzer, while simultaneously measuring the interior chamber temperature, exterior ambient temperature, and PAR (Figure 1). The footprint of the chamber was 50 cm x 50 cm ($\approx 0.25$ $m^2$) with a total volume of 0.133 $m^3$. Photosynthesis measurements were calculated based on the total canopy area of each 10 cm diameter pot. To safeguard against chamber leakage, foam and rubber gaskets were installed at the connecting points of the top and base modules. Four circulating fans were installed inside of the top module to provide adequate air mixing. For this study, $CO_2$ fluxes were calculated using a temporal model which analyzed $CO_2$ concentration changes within the closed system environment of the chamber (Soba et al. 2020). Photosynthesis measurements were collected at 0, 7, 14, 21, and 28 DAT.

Bermudagrass was watered approximately 6-hours prior to the photosynthesis measurements to ensure adequate moisture content. Bermudagrass pots were removed one at a time from the growth chamber and taken directly to the photosynthesis chamber located outside the building for $CO_2$ measurements. Pots were placed in direct sunlight in the chamber to eliminate any shading effects from the structural components of the chamber. The box was placed over the bermudagrass and clamped down to seal the chamber (Figure 1). Once sealed, measurements were initiated within one minute to restrict elevating temperatures within the chamber. Each $CO_2$ sequence lasted for 120 seconds, then the bermudagrass pot was removed and the next sample placed in the chamber. This process continued until all bermudagrass was analyzed. Measurements were collected between the hours of 1000 and 1400 central time (CT), with no measurements occurring after 1400 CT. Total time to collect $CO_2$ measurements, at each day of collection, was approximately two hours.

The captured $CO_2$ sequence data were then analyzed using the software Soil Flux Pro (LI-COR Biosciences, Lincoln, NE, USA). This software fits a linear regression line to the $CO_2$ sequence data collected within the chamber and calculates a slope of the trend line that is equivalent to the gross photosynthesis value. Accuracy of each measurement was then assessed by calculating the $R^2$ values and normalized sum of square residuals. In order to eliminate an increase in errors associated with chamber closure, the first 10 seconds of each two-minute $CO_2$ sequence were removed (Soba et al. 2020).

**Pigment Determination for Bermudagrass.** Pigments were extracted to quantify chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total carotenoids (β-carotene and xanthophylls) according to methods used by Lictenthaler (1987). A subsample of previously collected bermudagrass shoots weighing approximately 0.05 g was placed in a mortar and pestle with approximately 2 mL of 99.5% acetone (dimethyl ketone; $CH_3COCH_3$; VWR Chemical, Radnor PA). Each sample was ground for 1 to 2 minutes to ensure total release of the pigments from the leaf tissue, and then transferred to a 15 mL ultra-high-performance centrifuge tube (VWR International LLC, Radnor PA). The mortar and pestle were rinsed using acetone and the resulting rinsate was also transferred to the centrifuge tube. Acetone was then added to the centrifuge tube to bring the total volume up to 10 mL. The tubes were sealed and placed in a compact centrifuge (Corning LSE, Corning, NY) fitted with a 12-tube capacity rotor and centrifuged at 3000 g for 10 min. Once centrifuging was completed, a 300µL aliquot of the supernatant was transferred to a 96 well, clear, flat bottom Costar assay spectrophotometer plate (Corning Inc., Kennebunk, ME) and analyzed spectrophotometrically using a Biotek µQuant microplate reader (Biotek, Winooski, VT) mated with the Gen5 software package (Biotek, Winooski, VT). Samples were analyzed at the absorbance spectra of 661.6 nm (A661.6, Chl *a*), 644.8 nm (A644.8, Chl *b*), and 470.0nm (A470, Carotenoids). Pigment concentrations were calculated using the pure solvent 100% acetone formulae provided below (Lictenthaler, 1987):

$$\text{Chl } a = 11.24A_{661.6} - 2.04A_{644.8}$$

$$\text{Chl } b = 20.13A_{644.8} - 4.19A_{661.6}$$

$$\text{Total Carotenoids} = \frac{1000A_{470} - 1.90(\text{Chl } a) - 63.14(\text{Chl } b)}{214}$$

where final pigment concentrations are in μg $mL^{-1}$ of extract solution, and A is the absorbance value for the respective pigments at 470, 644.8, and 661.6nm. Reported values of pigment concentration were converted to mg $g^{-1}$ fresh weight (FW) of bermudagrass leaf tissue.

**Statistical Analysis.** Topramezone and nutrient treatments were considered the main effects of a factorial design with 3 replications of each treatment combination, arranged in a completely randomized design in each growth chamber set to the desired season temperature. All experiments were repeated twice in time. Data from non-treated bermudagrass were excluded where appropriate to help stabilize variance. Data were initially subjected to analysis of variance (PROC ANOVA) ($P = 0.05$) to determine the main effects and test interactions. Means were separated using Fisher's Protected LSD within the PROC GLM function of SAS 9.4 (SAS Institute, Cary, NC, 27513). Correlations of data were run using PROC CORR in SAS 9.4.

## Results and Discussion

**Bleaching.** For bleaching, none of the treatment interactions were significant ($P = 0.26 – 0.56$) except for the treatment-by-season-by-DAT interaction ($P < 0.0001$). Data were separated based on season and pooled across runs. Data were separated by DAT to aid in analysis and discussion. Data for interaction of season and treatment on bleaching is shown in Figure 2. Correlation data comparing bleaching with chlorophyll *a* ( $r = -0.42547$) were moderately negative and statistically significant ($P < 0.0001$) (Table S4). These correlations suggest that when bleaching increases, a decrease in chlorophyll *a* should be expected. All other variables were weakly correlated (Table S4). Data for the non-treated control and nutrient only treatments were removed from the bleaching portion of the study, since all bleaching ratings were equal to zero for all rating dates.

Bleaching severity was affected by season (Figure 2), with damage appearing earlier in the summer season. Increases in bleaching severity are likely due to longer daylengths which are coupled with high light intensity. Bermudagrass grown in cooler temperatures had bleaching symptoms at 14 DAT, which disappeared by 28 DAT, while warmer temperatures had bermudagrass with bleaching at 7 DAT, which lasted until 21 DAT (Figure 2). Thus, both temperature groups resulted in bleaching symptoms which lasted for approximately 14 days, with symptoms delayed in the fall.

In the fall regime, bermudagrass bleaching was higher at 14 and 21 DAT in bermudagrass to which topramezone plus FeDTPA/N had been applied. While the intent of this work was to study solely Fe chelate (FeDTPA), the product used (Sprint 330) was changed (from that used in earlier studies) with the addition of 7% nitrogen. This N addition was not reflected on the research product label (not a commercial label) that was mailed, and so N was added with the iron chelate. Even though an agronomically small rate, this addition of N to the iron source effectively removed the bleaching reduction potential of the chelated iron observed in earlier studies (Boyd et al. 2016; 2018) and increased overall bleaching (Figure 2). This only happened in the fall, and not in the summer. Differences in bleaching as a function of N due to season could be the result of the bermudagrass growing far more actively in the summer than fall, with average clipping yields of 0.6 g fresh wt harvest$^{-1}$ in the fall, versus 1.0 g in the summer (averaged over all sampling dates and treatments). Simply, the small amount of added N did not affect the more actively growing bermudagrass.

Increases in bermudagrass bleaching as a function of N addition have been shown previously, as the addition of ammonium sulfate (1.7 kg N $ha^{-1}$) to topramezone increased bermudagrass bleaching over that observed when topramezone was applied alone (Boyd et al. 2016). In other work, iron sources that also contained N (ranging from 0.1 to 4.6 kg N $ha^{-1}$) created increased bermudagrass bleaching when combined to topramezone, when compared to bleaching created by application of topramezone combined with iron sources lacking N (Boyd et al. 2018). For comparison, the amount of N applied in this study was lower (0.9 kg N $ha^{-1}$) than applied in previous work, but similar results occurred.

Inclusion of N and/or Fe in herbicide sprays has produced varying results, usually as a function of the herbicide. When ammonium sulfate was applied as a surfactant at low rates with an herbicide, an increase in herbicide uptake and translocation were observed (Jordan et al. 1989; Maschoff et al. 2000). When a chelated iron plus N product (same as the one used in this work) was tank-mixed with bispyribac-sodium chlorosis of creeping bentgrass was masked, at any rate of herbicide application, with consistent control of annual bluegrass (*Poa annua* L.) (McDonald et al. 2006). Varying with Fe source, the addition of $FeSO_4$ to MSMA tank mixes reduced bermudagrass injury, but also reduced crabgrass control (Massey et al. 2006). However, when FeDTPA was the Fe source neither antagonism nor safening was found (Massey et al. 2006). Thus, differences in response are likely a function of herbicide chemistries and mode of action.

The topramezone mixtures which included $ZnSO_4$ or $FeSO_4$ produced significantly less bermudagrass bleaching, when compared to that bleaching produced when topramezone was applied alone. Application of either zinc sulfate or iron sulfate produced bermudagrass with acceptable bleaching ratings of less than 20% for the duration of all trials. The most effective treatment was the inclusion of $FeSO_4$. When added to topramezone bermudagrass bleaching was reduced to less than 5%, across all rating dates and seasons (Figure 2). These results agree with previous studies, which reported reductions in herbicide phytotoxicity when applied in combination with iron (Flessner et al. 2017; Johnson and Carrow 1995; Massey et al. 2006; McCullough and Hart 2009).

**Whole Canopy Photosynthesis Analysis.** For measurements of photosynthesis, the interaction of treatment-by-season was the only significant interaction ($P < 0.0001$). Data were pooled across runs and separated by season. Corresponding data are found in Figure 3. Correlation data comparing photosynthesis analysis with clipping yield ($r = -0.35616$) were moderately negative and statistically significant ($P < 0.0001$) (Table S4). These correlations suggest that photosynthesis measures higher when clipping yield is lower and vice versa. All other correlations were found to be weakly correlated with photosynthetic analysis and will not be discussed (Table S4).

Application of topramezone alone always decreased photosynthesis, when compared to the non-treated control. This occurred in both seasons and was generally significant from 7 to 14 days after treatment in the fall, and at 7 DAT in the summer (Figure 3).

In the fall, the addition of either FeDTPA/N or $ZnSO_4$ to the topramezone had either no effect ($ZnSO_4$) or further reduced (FeDTPA/N) photosynthesis, as compared to that measured in the topramezone-only treatment. Addition of the FeDTPA/N had little beneficial effect on photosynthesis (Figure 3), and in the fall the inclusion of FeDTPA/N actually reduced photosynthesis below that measured in the topramezone-only treatments. As with the bleaching data, the addition of the small amount of N appears to antagonize safening abilities of this iron source (Jordan et al. 1989; Maschoff et al. 2000).

In the summer the addition of $ZnSO_4$ to the topramezone did improve photosynthesis (as compared to the topramezone-only treatment) but the addition of $ZnSO_4$ alone also had this effect. So, although the addition of $ZnSO_4$ to topramezone did reduce overall bleaching of bermudagrass (Figure 2), protected photosynthetic efficiency was not observed in the fall, and improvements in photosynthesis in the summer occurred when $ZnSO_4$ was applied, regardless of topramezone inclusion. So, inclusion of $ZnSO_4$ with topramezone is not reducing bleaching of bermudagrass by some effect on photosynthesis. While Zn does play a role in photosynthesis through the Zn-containing enzyme carbonic anhydrase, for the conversion of bicarbonate to carbon dioxide (Hull 2001), there were no additional benefits in photosynthesis due to the foliar application of zinc, in this study.

The only safener treatment to consistently protect photosynthesis, as compared to the non-treated control, was the addition of $FeSO_4$ to the topramezone. When $FeSO_4$ was applied with topramezone, photosynthesis was measured at the same levels as that measured in the non-treated controls. This was significant at 14 and 21 DAT in the fall, and at every measurement date in the summer (Figure 3).

**Pigment Analysis.**

**Chlorophyll *a*** – All interactions were not significant ($P = 0.43-0.81$) except for the interaction of treatment-by-season ($P < 0.0001$). Correlations comparing chlorophyll *a* with chlorophyll *b* were moderately positive ($r = 0.56308$) and statistically significant. ($P < 0.0001$) (Table S4). Data were pooled across runs and separated by season for discussion. Individual nutrients were also separated in the figures to aid in clarity. Corresponding data is presented in Figure 4.

In both the fall and summer regimes, bermudagrass chlorophyll *a* concentration was reduced whenever topramezone was part of a treatment (Figure 4). Reductions in chlorophyll *a* have been shown with other herbicide chemistries, including mesotrione (McCurdy et al. 2008) and another HPPD inhibitor, isoxaflutole (Bhowmik and Drohen, 2001). Decreases in chlorophyll *a* due to topramezone application are also a function of herbicide rate, with higher rates (18, 25, and 38 g ai ha$^{-1}$) creating a greater decrease in chlorophyll *a* than observed in this work (Brosnan et al. 2011). Destruction of chlorophyll by reactive oxygen species (ROS) is a result of topramezone application to bermudagrass. Topramezone effectively prevents the formation of a-tocopherol and carotenoids, which both function as chlorophyll photoprotectors by quenching excess energy from ROS (McCurdy et al. 2008). Since the ROS are no longer regulated, destruction of nearby chlorophyll results in the appearance of bleaching symptoms (Havaux 1998; Sandmann and Boger 1997; Trebst et al. 2002).

The addition of $ZnSO_4$ or FeDTPA/N to the topramezone never affected concentrations of chlorophyll *a*, and response curves (FeDTPA/N or $ZnSO_4$ with or without topramezone), in both fall and summer seasons, were essentially the same. The addition of $FeSO_4$, FeDTPA/N or $ZnSO_4$ (without topramezone) always produced levels of chlorophyll *a* equal to those measured in the non-treated control, at every sampling date and in both seasons (Figure 4).

In both the fall and summer, the addition of $FeSO_4$ to topramezone often increased chlorophyll *a* concentration as compared to those measured in the topramezone-only treatment (Figure 4). This was significant at 14 DAT in the fall, and at 7 and 14 DAT in the summer. Although increases in chlorophyll are often associated with foliar Fe application, not all turfgrass research has shown this. For example, foliar application of Fe did not increase chlorophyll *a*:*b* concentrations, when applied to a mixed sward of supina and Kentucky bluegrass (*Poa supina/Poa pratensis* L.) (Stier and Rogers 2001). Similarly, fertilization of bermudagrass with foliar Fe (as FeDTPA) produced no increase in total chlorophyll (White and Schmidt 1989). Alternatively, others found that chlorophyll content of Kentucky bluegrass (both *a* and *b*) increased with elevated levels of Fe in a hydroponic solution (Lee et al. 1996).

As the effects of topramezone application dissipated, concentrations of chlorophyll *a* in bermudagrass treated with topramezone, topramezone plus $ZnSO_4$, or topramezone plus FeDTPA/N increased at 21 and 28 DAT, and were then equal to levels measured in the non-treated control treatment. The only treatment which did not follow this pattern was the topramezone plus FeDTPA/N treatment, which delayed recovery of chlorophyll *a* in the bermudagrass when compared to that of bermudagrass to which only topramezone had been applied (21 DAT, fall only). This delay in recovery indicated how injurious the application of topramezone plus FeDTPA/N was to the bermudagrass growing in cooler conditions. By 28 DAT, all bermudagrass chlorophyll *a* concentrations were the same across all treatments, indicating recovery (Figure 4).

**Chlorophyll *b*** – All interactions were not significant (P = 0.20-0.99). The main effect of treatment was significant (P = 0.0008). All data were pooled across season and runs. Data are presented by the treatment main effect and separated by nutrient for graph clarity (Figure 5). Correlations comparing chlorophyll *b* with chlorophyll *a* were moderately positive (r = 0.56308) and statistically significant ($P < 0.0001$). All other variables were weakly correlated and will not be discussed (Table S4).

Levels of chlorophyll *b* were largely unaffected by treatment (Figure 5). Chlorophyll *b* data had fairly large standard errors, and differences due to treatment were few (Figure 5). In general chlorophyll *b* decreased over time in all treatments, and increased after 14 DAT. Since even the non-treated controls had this same behavior, the increase in chlorophyll *b* once past 14 DAT appears to be unrelated to treatment. The only outlier to this general response was when $FeSO_4$ or $ZnSO_4$ was added, without inclusion of topramezone. In those two cases, at 21 DAT, chlorophyll *b* was higher, when compared to any other treatments (the non-treated control and any containing topramezone). This lack of treatment response in chlorophyll *b* has been shown elsewhere, in similar herbicide families. Application of HPPD inhibitors increased oxidative stress, which had a greater effect on the concentration of chlorophyll *a* than chlorophyll *b* (Elmore et al. 2011, 2012; McCurdy et al. 2008).

Chlorophyll *b* functions as an accessory pigment to chlorophyll *a* to expand the spectrum of light that a plant is able to capture during photosynthesis. Chlorophyll *b* concentrations are often found to be approximately one-half to one-third of the concentration of chlorophyll *a*, and that was observed in this work. In our study, initial chlorophyll *a* concentrations ranged from 1.8 to 2.8 mg $g^{-1}$ fresh weight (FW), while chlorophyll *b* concentrations ranged from 1 to 1.3 mg $g^{-1}$ FW, respectively. McCurdy et al. (2008) reported similar ratios of chlorophyll *a* to chlorophyll *b* with ranges of 2.9:1 to 3.1:1. Work by Brosnan et al. (2011) reported only total chlorophyll concentrations, but overall concentrations were similar to those reported here.

Another factor affecting the concentration of chlorophyll *a* and *b* is environmental conditions. One study showed that under shaded conditions or lower light intensity, the concentration of both chlorophyll *a* and *b* were higher than those observed during full sun or higher light intensities (Aldahir 2015). These findings suggest that higher density of chlorophyll within the leaf tissue is required under lower light conditions to maximize photosynthetic efficiency. Seasonal effects were tested in our study, which included temperature, and had no effect on chlorophyll *b*. Similar findings were reported by McCurdy et al. (2008) that showed no differences in chlorophyll *b* concentrations for temperatures of 18 and 32ºC. Additional work also showed similar results to our study (Elmore et al. 2011, 2012; Grossman and Ehrhardt 2007).

**Total Carotenoids** – None of the interactions were significant except for the treatment-by-season interaction ($P < 0.0001$). Correlations comparing total carotenoid concentrations with chlorophyll *a* were strongly correlated and statistically significant ($P < 0.0001$) (Table S4). These correlations suggested that as carotenoid concentrations increased, the chlorophyll *a* also increased. All other variables were weakly correlated with total carotenoid concentration. Data were pooled across runs and separated by season. Figures have been separated by nutrient for clarity. Treatment-by-season data is presented in Figure 6. Total carotenoids are a quantification of both *B*-carotene and xanthophyll pigments (Lictenthaler 1987).

The effect of topramezone application was greater in the fall than the summer, with largest differences in carotenoids due to treatment found at 14 DAT in the fall, and at 7 DAT in the summer (Figure 6). Over both seasons, the application of topramezone decreased total carotenoids for 7 (summer) to 14 (fall) days after application, and this was often significantly different from the carotenoid concentrations measured in the non-treated control plants. Over both seasons, application of topramezone reduced total carotenoids (compared to the control) in 9 of the 30 times that data was collected, all in the first 14 days of data collection. At 21 and 28 DAT total carotenoid concentrations in bermudagrass to which topramezone was applied was always equal to that measured in the non-treated control (Figure 6). Thus, carotenoid concentrations in either season were similar to bleaching results. As bleaching symptoms increased, the pigment concentrations of carotenoids decreased (through 14 DAT). Likewise, as bleaching symptoms decreased, an increase in carotenoid concentrations were observed.

While there were instances in which the application of $FeSO_4$, FeDTPA/N or $ZnSO_4$ affected total carotenoid concentrations, these were inconsistent. In the fall, the addition of FeDTPA/N or $ZnSO_4$ to topramezone never affected carotenoid concentrations differently than that measured in the topramezone-only treatments (Figure 6). This was also true in the summer, with the exception of the 7 DAT data for topramezone + FeDTPA/N, which had higher carotenoid concentrations than those measured in the topramezone-only treatment. So, adding FeDPTA/N or ZnSO4 to topramezone had little effect on total carotenoids. Adding only FeDTPA/N or $ZnSO_4$ (no topramezone) also had little effect, and in both the summer and fall scenarios total carotenoids in those treatments was almost always equal to those measured in the non-treated controls. Previous research has also shown that increasing rates of foliar Fe had no response on total carotenoids, as measured in Kentucky bluegrass (Lee et al. 1996).

The application of $FeSO_4$ with topramezone did increase carotenoid concentrations, when compared to those measured when only topramezone was applied (Figure 6). This was significant at 14 DAT (fall) and 7 DAT (summer), which matches the point at which this same treatment most protected the bermudagrass against bleaching. As with the other nutrient sources, application of $FeSO_4$ did not increase total carotenoid concentrations beyond that measured in the non-treated control treatments.

Lack of change in total carotenoids in turfgrasses as affected by herbicides has been found previously. In one study, carotenoid concentrations did not significantly decrease following application of mesotrione to perennial ryegrass (McCurdy et al. 2008). In other work, total carotenoid concentration decreased shortly after application of topramezone, with a quick recovery following the 7 DAT rating (Brosnan et al. 2011). Those results are similar to those found here, except in the fall treatments it took 14 DAT for recovery.

**Clipping Yield** – None of the interactions were significant except for treatment-by-season ($P = 0.02$). Correlations comparing clipping yield with photosynthetic efficiency were moderately negative ($r = -0.35616$) and statistically significant ($P < 0.0001$) (Table S4). All other correlations were weakly correlated and will not be discussed (Table S4). Data were pooled across runs and separated based on season (Figure 7). All of the figures were separated by nutrient as well, for graph clarity.

Application of topramezone sometimes reduced clipping yields, but it was rarely significant from that measured in other treatments. There were three instances when any treatment affected clipping yield, and none of these responses were consistent. These were: 1) at 21 DAT (Fall), bermudagrass to which FeDTPA/N had been applied had a lower clipping yield (than any other treatment), 2) at 21 DAT (Fall) bermudagrass to which topramezone + $FeSO_4$ had been applied had greater clipping yield than that from the topramezone-only treatment, and, 3) bermudagrass to which only $FeSO_4$ had been applied had greater clipping yield than that of the non-treated control (Summer, 21 DAT). As shown previously, application of Fe is often associated with improvements in turfgrass color, but not consistently leaf growth (Lee et al. 1996; Zhang et al. 2002).

Yield of all clippings was greater in the summer regime than fall, simply a factor of warmer temperature and longer daylength for the warm season bermudagrass. Bleaching of the bermudagrass from application of topramezone did not appear to dramatically affect turfgrass clipping yield, with the exception of a few measurement dates during the summer regime. For example, when bermudagrass was sprayed with topramezone + $FeSO_4$ clipping yield dropped from 0 DAT to 7 DAT, but then recovered to almost initial levels at 14 DAT (Figure 7). Bermudagrass treated with either $ZnSO_4$ alone or topramezone + $ZnSO_4$ also had this response, and it was also observed in the topramezone + FeDTPA/N and FeDTPA/N only treatments. No such behavior was ever observed in the Fall regime. When viewed with error bars as a reference, differences and responses largely appear variable, and unrelated to treatment.

## Discussion

Previous research has clearly shown that application of $FeSO_4$ safens hybrid bermudagrass to which the HPPD inhibiting herbicide topramezone has been applied. The result is a lessening of the bleaching symptoms, with recovery from any bleaching also occurring in a quicker time period. Unfortunately, the inclusion of N in the other Fe source (FeDTPA) renders conclusions about the utility of that product for safening somewhat moot, except to demonstrate that if turfgrass managers are seeking an Fe-based safener they should carefully read the label before using. This is borne in the literature, where one paper found negative consequences of using the same brand of FeDTPA used here, but the lack or presence of N in that product was not mentioned (Massey et al. 2006).

Since topramezone is an HPPD inhibitor which targets the photoprotective pigments of carotenoids in susceptible plant species, fluctuations in concentration values as a result of herbicide application were expected. The six primary components of carotenoids are comprised of antheraxanthin, violaxanthin, zeaxanthin, lutein, beta-carotene, and neoxanthin (Sandmann 2001). One of the main functions of carotenoids includes the transmitting of photons captured by light harvesting pigments in the chloroplast structure that were previously unabsorbed by the chlorophyll molecules during photosynthesis (Brosnan et al. 2011; Niyogi 1999; Niyogi et al. 1997; Polle et al. 2001). Other important functions include the quenching of free radical ROS, photoprotection, and the dissipation of excess energy by means of heat and light energy (Demmig-Adams 1990; Demmig-Adams et al. 1996; Frank and Cogdell 1996). Reductions in carotenoid concentrations are associated with the inhibition of HPPD, which increases phytoene accumulation due to incidental inhibitions of phytoene desaturase (Mayonado et al. 1989; McCurdy et al. 2008; Soeda and Uchida 1987). Phytoene functions as a precursor to lycopene, which forms either alpha-carotene followed by lutein, or beta-carotene followed by production of xanthophyll pigments including antheraxanthin, zeaxanthin, or violaxanthin (Croce et al. 1999). Earlier research showed that increased amounts of zeaxanthin decreased the light harvesting ability of photosynthetic antennae (Baroli et al. 2003; Croce et al. 1999). The xanthophyll pigments work together to expend the excitation energy of triplet-state chlorophyll, in turn impeding the formation of singlet oxygen and other ROS. One of the most important functions of carotenoids is the quenching of excess energy provided during photosynthesis. These pigments operate as photoprotectors of the photosynthetic light harvesting components, and when inhibited produce severe injury and oxidation of the chlorophyll by ROS (Baroli et al. 2003; Croce et al. 1999; McCurdy et al. 2008).

In this work chlorophyll *a* and carotenoids were affected by the application of topramezone, with decreases in both up to 14 DAT, regardless of season (Figures 4 and 6). Concentrations of chlorophyll *b* were largely unaffected by the application of topramezone. Application of FeDTPA/N with the topramezone never affected photosynthesis, chlorophyll *a*, *b* or carotenoid concentrations, likely a consequence of the added nitrogen in the Sprint 330 commercial product. This deserves additional study, and the inclusion of Fe chelate sources that do not include nitrogen.

It appears that inclusion of $FeSO_4$ with topramezone safens the herbicide by protecting photosynthetic activity and maintaining concentrations of chlorophyll *a* and carotenoids. Since the role of iron in chlorophyll is well known (Rout and Sahoo 2015) this data supports the idea that added foliar Fe is increasing chlorophyll production in leaves damaged by topramezone application.

## Acknowledgments

The authors would like to thank Jane Farr and her team at the Auburn Plant Science Research Center for providing the potting, greenhouse, and growth chamber space used during the experiments. Special thanks go to Dr. Alvaro Sanz-Saez and his lab for providing the photosynthesis chamber used during this experiment. Additional thanks go to Bill Bryce and the Guertal lab for all of their help processing samples for pigment concentration analysis. Thanks also go to David Lawrence and his crew at the Auburn Turfgrass Research Unit for providing the bermudagrass plugs used during the experiment. No conflicts of interest have been declared.

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Table 1. Application details for growth chamber trials evaluating bermudagrass injury following topramezone application and nutrients. Auburn, AL.

| Application trial data | | | |
|---|---|---|---|
| Run name | Initiation dates | Ending dates | Season[a] |
| Summer 20-1 | 3/2/2020 | 3/30/2020 | Summer |
| Summer 20-2 | 3/27/2020 | 4/24/2020 | Summer |
| Fall 20-1 | 3/2/2020 | 3/30/2020 | Fall |
| Fall 20-2 | 3/27/2020 | 4/24/2020 | Fall |

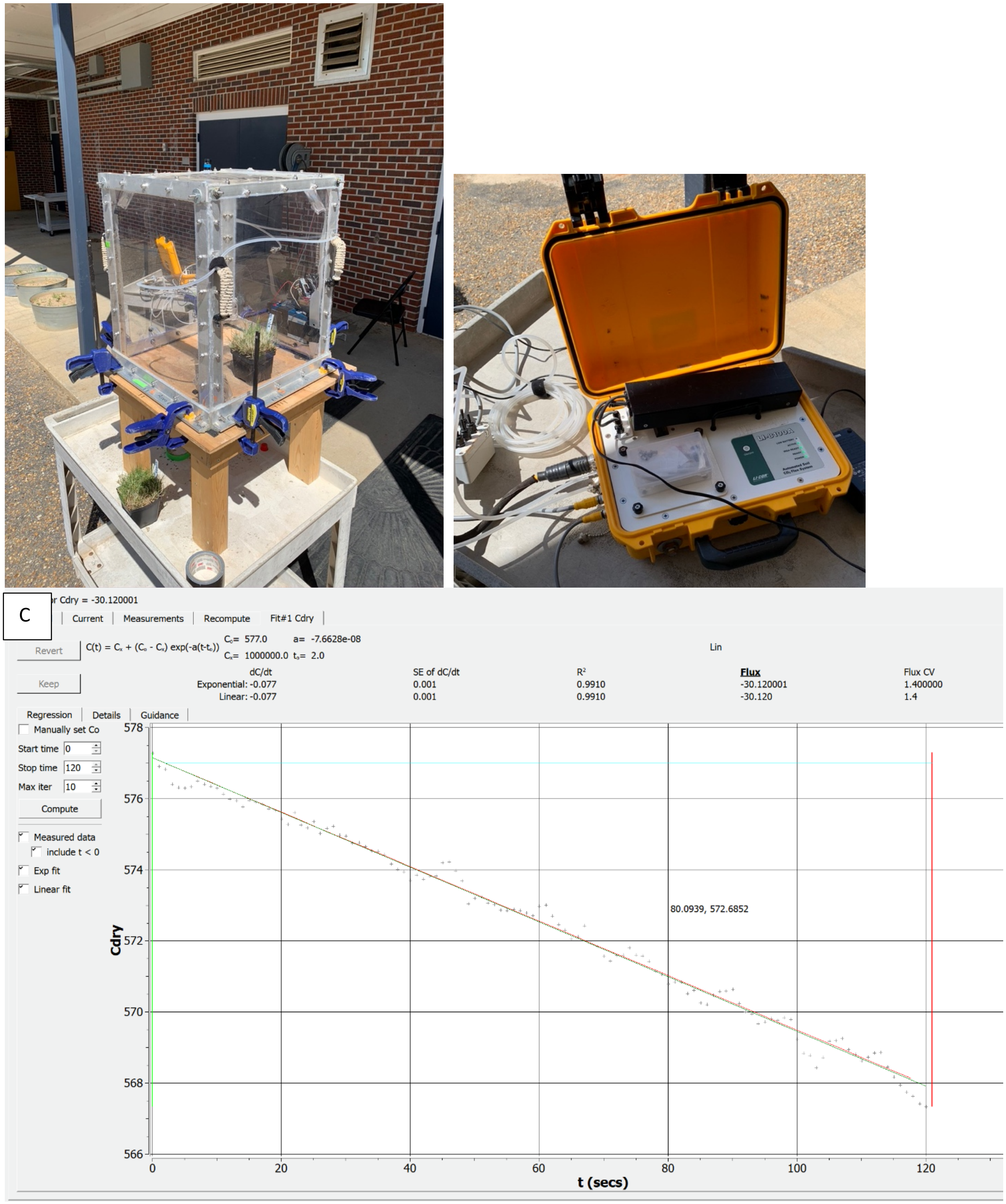
[a] Summer temperature settings were set at 31.9°C for 14 daytime hours, and 20.8 °C during night time hours to simulate conditions found in Auburn, AL in July/August. Fall temperature settings were set at 23.9°C for 11.5 daytime hours and 12.2°C during night time hours to simulate conditions found in Auburn, AL in October/November.

Table 2. List of treatments and rates used for topramezone and micronutrient growth chamber trials, applied to bermudagrass in 2020, Auburn, AL

| Active ingredient[a] | Trade name | Manufacturer | Topramezone rate g a.i. $ha^{-1}$ | Additive rate kg nutrient $ha^{-1}$ | N applied kg $ha^{-1}$ |
|---|---|---|---|---|---|
| Topramezone | Pylex® | BASF<br>Research Triangle Park, NC | 12.3 | --- | --- |
| Topramezone<br>FeDTPA/N [b] | Sprint®330 | BASF<br>Research Triangle Park, NC | 12.3 | 1.22 | 0.9 |
| Topramezone<br>Zinc sulfate | Zinc sulfate<br>($ZnSO_4$) | VWR<br>Radnor, PA | 12.3 | 2.08 | --- |
| Topramezone<br>Ferrous sulfate | Ferrous sulfate<br>($FeSO_4$) | Crown Technology Inc.<br>Indianapolis, IN | 12.3 | 1.22 | --- |
| FeDTPA/N | Sprint®330[b] | VWR<br>Radnor, PA | --- | 1.22 | 0.9 |
| Zinc sulfate | Zinc sulfate<br>($ZnSO_4$) | VWR<br>Radnor, PA | --- | 2.08 | --- |
| Ferrous sulfate | Ferrous sulfate<br>($FeSO_4$) | Crown Technology Inc.<br>Indianapolis, IN | --- | 1.22 | --- |
| Non-Treated | N/A | N/A | --- | --- | --- |

[a] All treatments containing topramezone were mixed with methylated seed oil (MSO; Alligare, Opelika, AL) at 0.5% v $v^{-1}$ in 1L bottles.

[b] The product used in this research contained 7% water soluble nitrogen. The specific source of nitrogen used was not clarified by the manufacturer.



r Cdry = -30.120001

$C(t) = C_x + (C_o - C_x)\exp(-a(t-t_o))$

$C_o$= 577.0   a= -7.6628e-08

$C_x$= 1000000.0   $t_o$= 2.0

|  | dC/dt | SE of dC/dt | R² | **Flux** | Flux CV |
|---|---|---|---|---|---|
| Exponential: | -0.077 | 0.001 | 0.9910 | -30.120001 | 1.400000 |
| Linear: | -0.077 | 0.001 | 0.9910 | -30.120 | 1.4 |

☐ Manually set Co

Start time 0

Stop time 120

Max iter 10

☑ Measured data

☑ include t < 0

☑ Exp fit

☑ Linear fit

Figure 1. (A) Photographs of photosynthesis chamber and (B) LI-8100 $CO_2$ analyzer used in this experiment. (C) Example output of $CO_2$ measurement using the Soil Flux Pro software (LICOR Biosciences, Lincoln, NE). Slight variation can be seen from zero until 10s mark, so first 10 seconds of each measurement were removed. Linear regression line represents the slope of the trend line which is equivalent to the gross photosynthesis value, with time (t) shown in seconds, and the $CO_2$ concentration value (Cdry) representing change of $CO_2$ within the chamber.

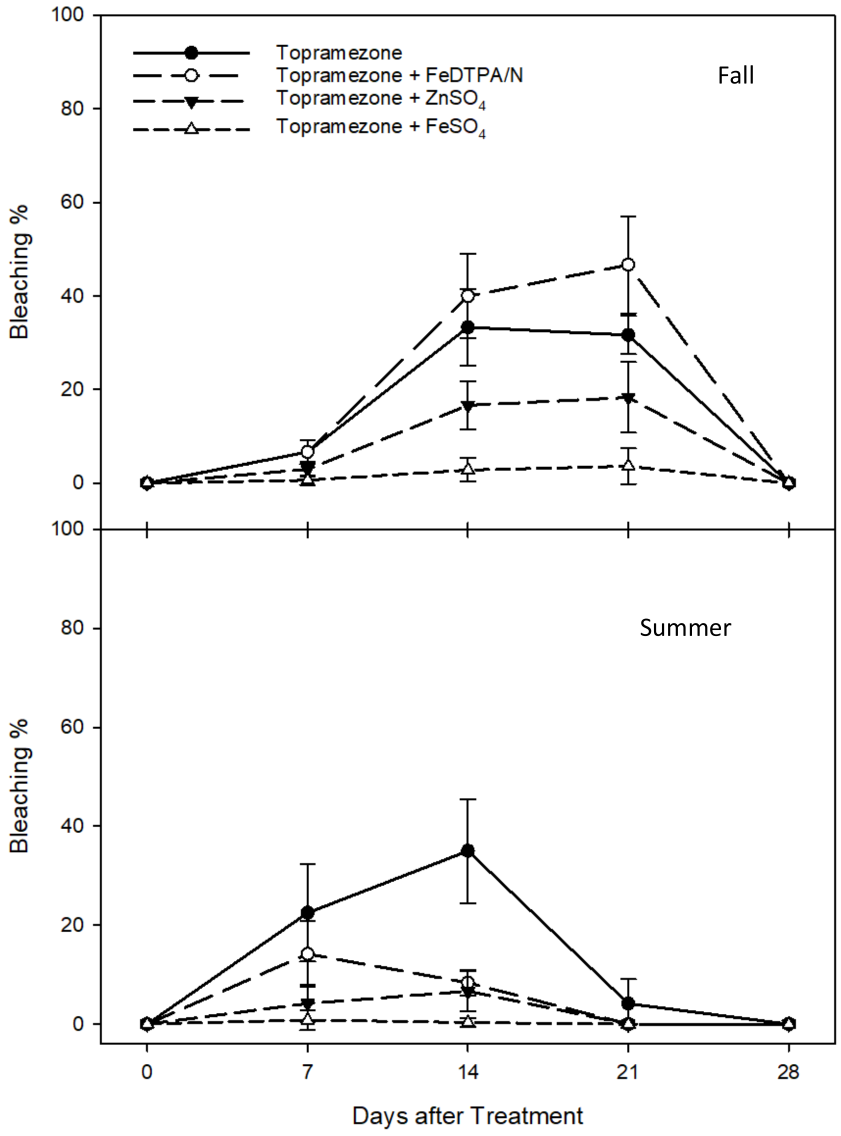


Figure 2. Visually-estimated bleaching percentage of bermudagrass following topramezone plus nutrient treatments at 0, 7, 14, 21, and 28 days after treatment in a growth chamber study in Auburn, AL in 2020. Nutrients listed are chelated iron (FeDTPA/N), zinc sulfate ($ZnSO_4$), and iron sulfate ($FeSO_4$). Summer temperature settings were set at 31.9˚C for 14 daytime hours, and 20.8˚C during night time hours to simulate conditions found in Auburn, AL in July/August. Fall temperature settings were set at 23.9˚C for 11.5 daytime hours and 12.2˚C during night time hours to simulate conditions found in Auburn, AL in October/November. Error bars indicate standard errors of means, *n* = 6.

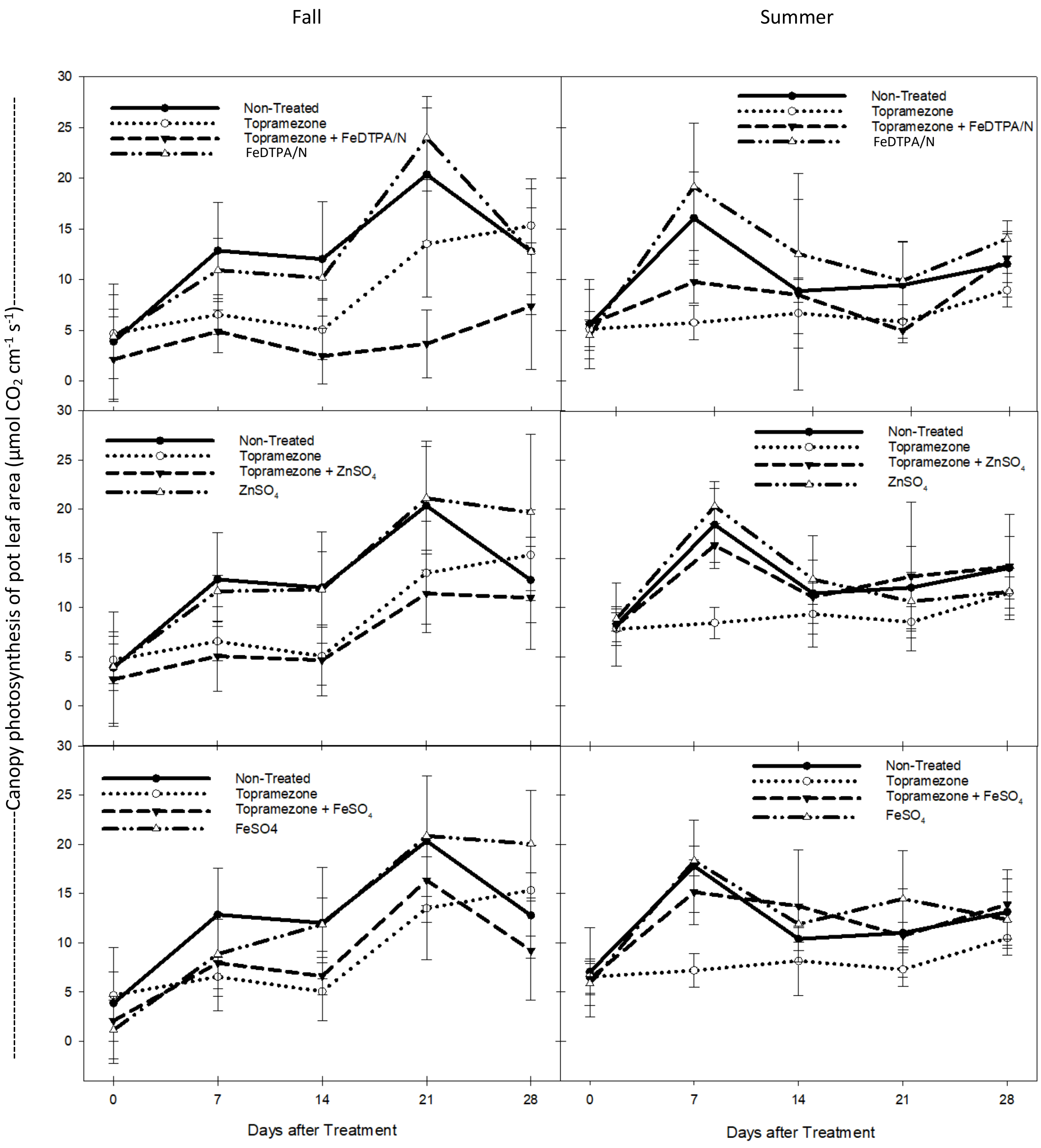


Figure 3. Whole canopy photosynthesis measurements of Tifway bermudagrass leaf area following topramezone plus nutrient treatments at 0, 7, 14, 21, and 28 days after treatment in a growth chamber study in Auburn, AL in 2020. Graphs are separated based on nutrient for clarity. Nutrients listed are chelated iron (FeDTPA/N), zinc sulfate ($ZnSO_4$), and iron sulfate ($FeSO_4$). Summer temperature settings were set at 31.9˚C for 14 daytime hours, and 20.8˚C during night time hours to simulate conditions found in Auburn, AL in July/August. Fall temperature settings were set at 23.9˚C for 11.5 daytime hours and 12.2˚C during night time hours to simulate conditions found in Auburn, AL in October/November. Error bars indicate standard errors of means, *n* = 6.

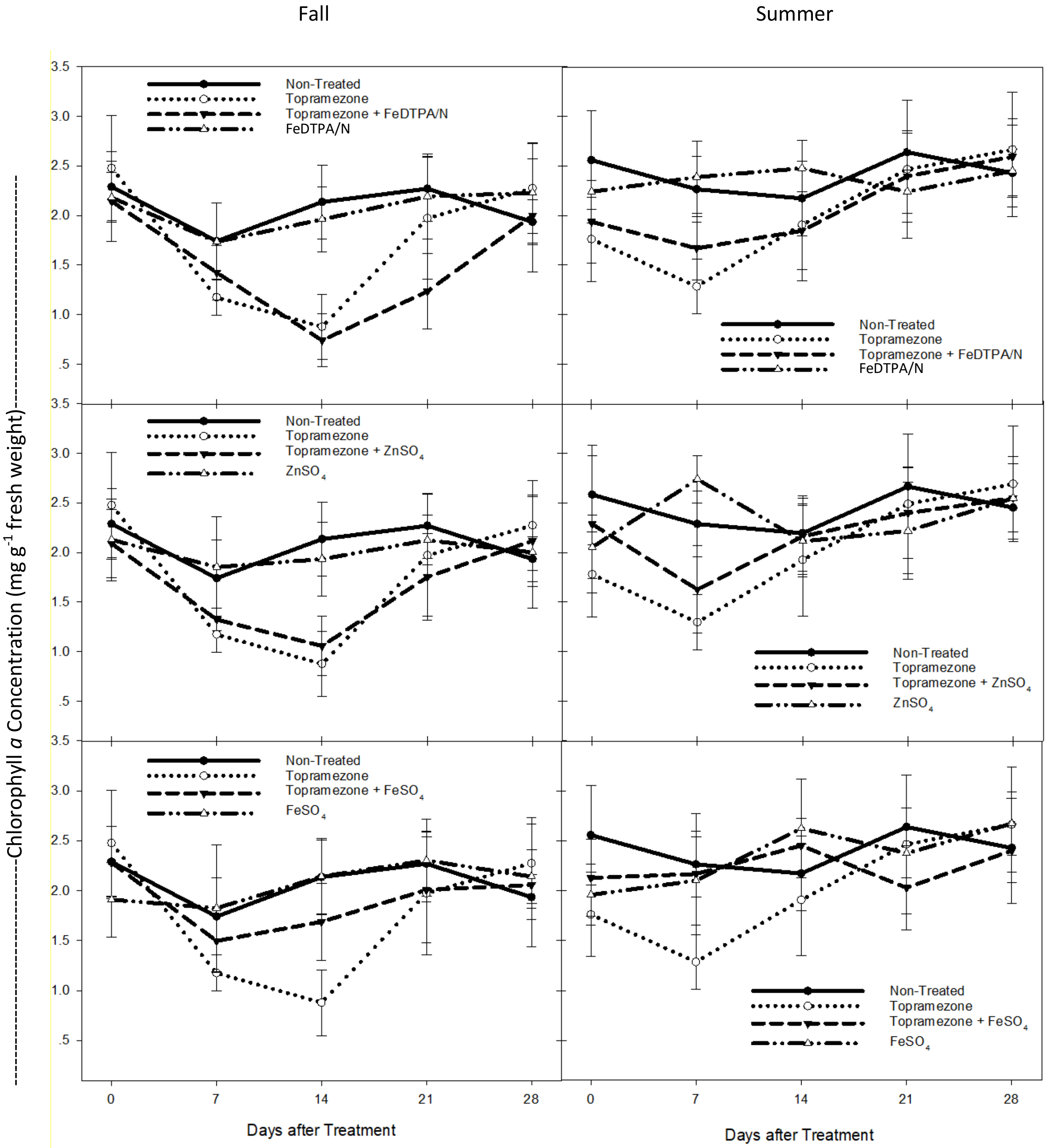


Figure 4. Spectral determination of chlorophyll *a* of Tifway bermudagrass following topramezone plus nutrient treatments at 0, 7, 14, 21, and 28 days after treatment in a growth chamber study in Auburn, AL in 2020. Graphs are separated based on nutrient for clarity. Nutrients listed are chelated iron (FeDTPA/N), zinc sulfate ($ZnSO_4$), and iron sulfate ($FeSO_4$). Summer temperature settings were set at 31.9 ˚C for 14 daytime hours, and 20.8 ˚C during night time hours to simulate conditions found in Auburn, AL in July/August. Fall temperature settings were set at 23.9 ˚C for 11.5 daytime hours and 12.2 ˚C during night time hours to simulate conditions found in Auburn, AL in October/November. Error bars indicate standard errors of means, n = 6.

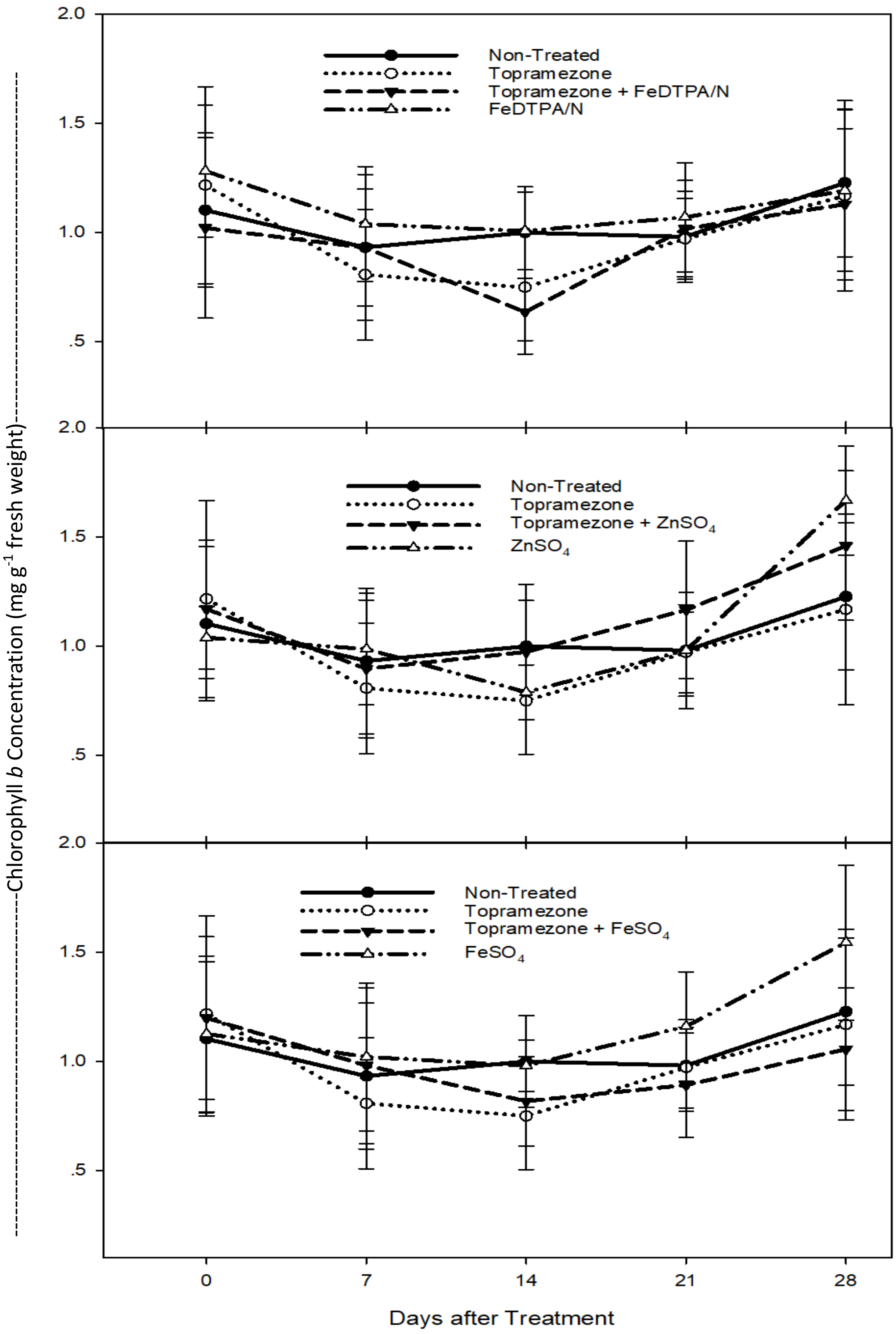


Figure 5. Spectral determination of chlorophyll $b$ in Tifway bermudagrass following topramezone plus nutrient treatments at 0, 7, 14, 21, and 28 days in a growth chamber study in Auburn, AL in 2020. Graphs are separated based on nutrient for clarity. Nutrients listed are chelated iron (FeDTPA/N), zinc sulfate ($ZnSO_4$), and iron sulfate ($FeSO_4$). Data pooled over 4 trials encompassing fall and summer seasons. Error bars indicate standard errors of means, $n$ = 12.

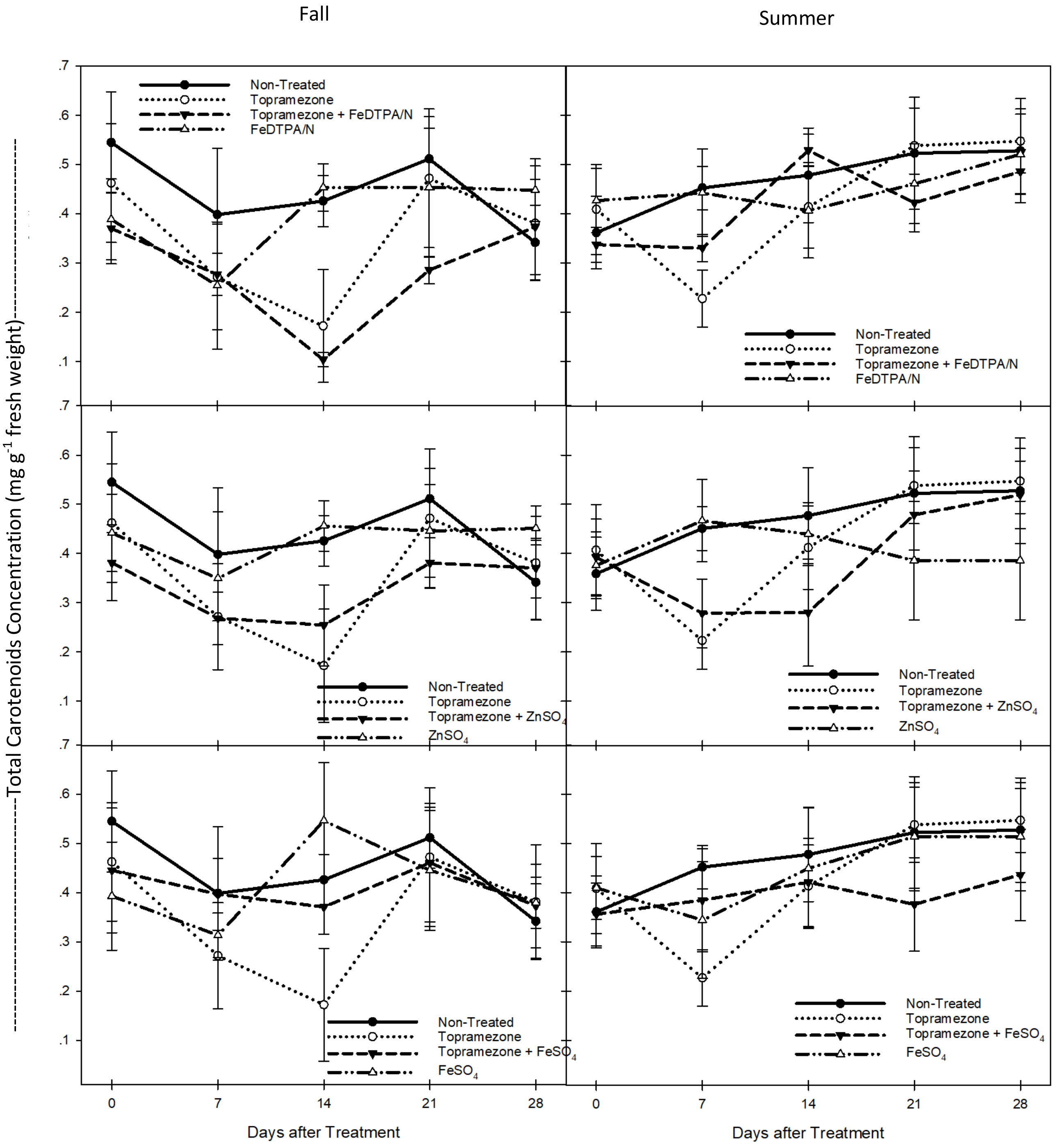


Figure 6. Spectral determination of total carotenoids of Tifway bermudagrass following topramezone plus nutrient treatments at 0, 7, 14, 21, and 28 days in a growth chamber study in Auburn, AL in 2020. Graphs are separated based on nutrient for clarity. Nutrients listed are chelated iron (FeDTPA/N), zinc sulfate ($ZnSO_4$), and iron sulfate ($FeSO_4$). Summer temperature settings were set at 31.9 ˚C for 14 daytime hours, and 20.8 ˚C during night time hours to simulate conditions found in Auburn, AL in July/August. Fall temperature settings were set at 23.9 ˚C for 11.5 daytime hours and 12.2 ˚C during night time hours to simulate conditions found in Auburn, AL in October/November. Error bars indicate standard errors of means, *n* = 6.

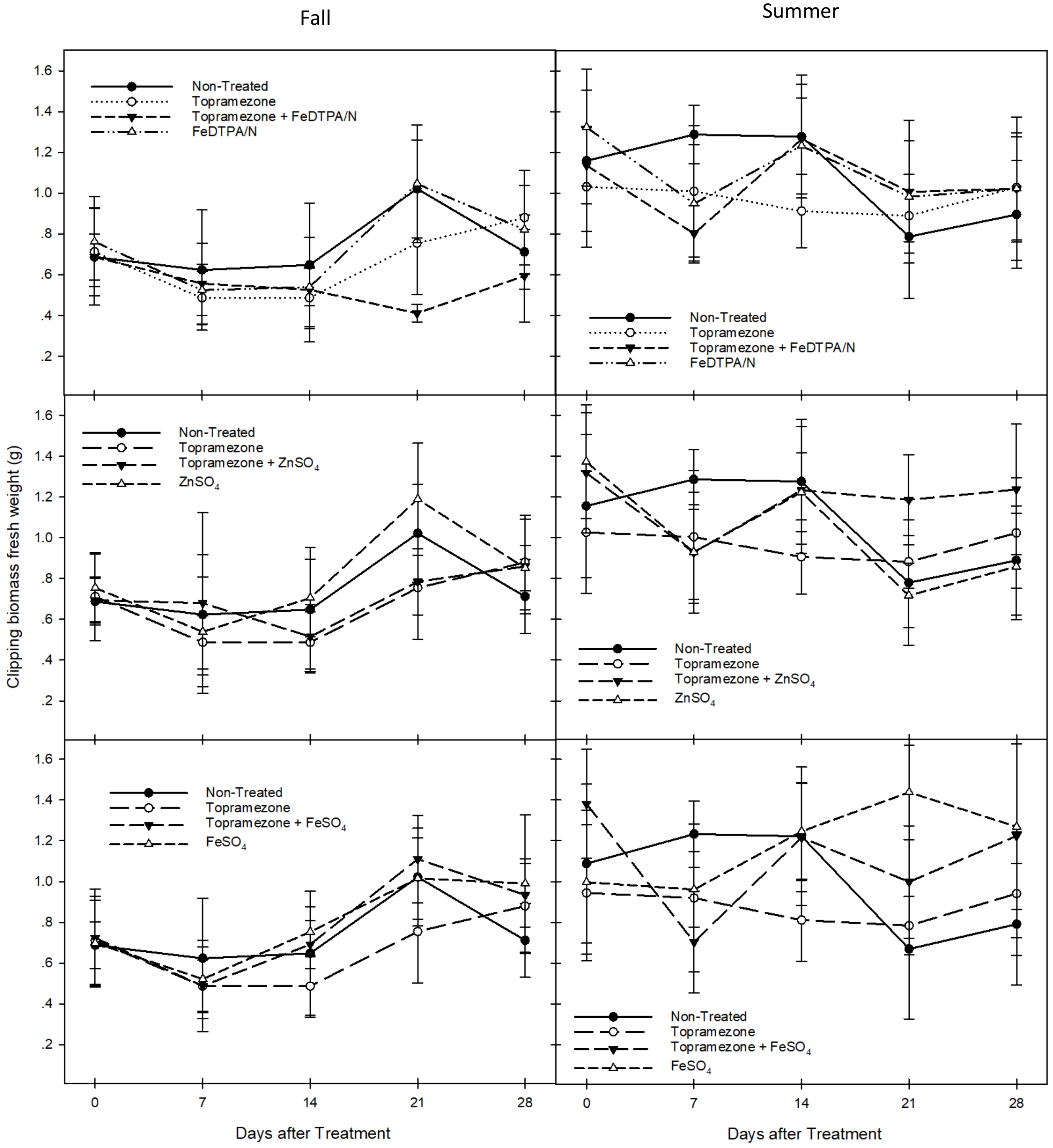


Figure 7. Clipping yield fresh weight biomass of Tifway bermudagrass following topramezone plus nutrient treatments at 0, 7, 14, 21, and 28 days after treatment in a growth chamber study in Auburn, AL in 2020. Graphs are separated based on nutrient for clarity. Nutrients listed are chelated iron (FeDTPA/N), zinc sulfate ($ZnSO_4$), and iron sulfate ($FeSO_4$). Summer temperature settings were set at 31.9°C for 14 daytime hours, and 20.8°C during night time hours to simulate conditions found in Auburn, AL in July/August. Fall temperature settings were set at 23.9°C for 11.5 daytime hours and 12.2°C during night time hours to simulate conditions found in Auburn, AL in October/November. Error bars indicate standard errors of means, *n* = 6.

# Conclusions

Bermudagrass is one of the most common turfgrasses utilized on golf courses, athletic fields, and home lawns in the US. Since most turfgrass areas are grown for aesthetic or recreational purposes, highly injurious herbicide applications are often discouraged, no matter how effective they are. Unfortunately, no herbicides have been found that can effectively control mature goosegrass without also causing phytotoxicity of the bermudagrass. Another issue is that the list of effective herbicides continue to decrease as regulatory pressure and herbicide resistance issues increase.

Topramezone is an excellent herbicide for difficult to control weeds at very low rates, but symptoms of bleaching and necrosis on the desired turfgrass are usually found to be unpleasing. Previous research has made attempts at safening on cool and warm season grasses and included growth regulators, herbicides, fungicides, and chemical safeners. This research built on the previous attempts and primarily focused on nutrient sources, while also included masking agents such as green turf paint and pigment, and triclopyr. Studies were initiated in 2015 and continued until 2020.

Results showed repeatedly that nutrient sources containing iron in the form of chelated iron FeDTPA or iron sulfate provided the highest levels of safening to bermudagrass. Zinc sulfate combined with topramezone and applied to bermudagrass also yielded great safening results. Since goosegrass control was not specifically tested with zinc sulfate, observed safening could potentially be an effect of antagonism as reported by other researchers using different herbicides in combination with zinc. Important findings showed that the addition of nitrogen to the topramezone mixture greatly increased bermudagrass bleaching and injury. When nitrogen was in solution with an iron product and mixed with topramezone, the safening effects of the iron product were either negated or greatly reduced. Common turfgrass products were intentionally used due to their ease of accessibility and popularity in the turfgrass market. It is very common for turfgrass managers, to mix multiple nutrients, fungicides, herbicides, growth regulators, etc. all in the same spray tank without really understanding how the different compounds could potentially antagonize one another. In the case of topramezone, mixing this herbicide with other compounds can completely antagonize its efficacy or increase overall phytotoxicity on desired turfgrasses. Environmental conditions also play a major role in the effectiveness of topramezone as well. It is very important to communicate the results of these studies so that turfgrass managers can make educated decisions, while also fighting against weed resistance issues.

# Appendix – Supplemental Information

Table S1. Means, standard deviations, and Pearson correlations for chapter 1 data.

| Variable | M | SD | NDVI | Bleaching | Necrosis | Clipping |
|---|---|---|---|---|---|---|
| **NDVI** | 0.61 | 0.11 | 1.00000 | | | |
| **Bleaching** | 10.59 | 19.99 | -0.38214** | 1.00000 | | |
| **Necrosis** | 18.59 | 26.66 | -0.79435** | 0.38947** | 1.00000 | |
| **Clipping** | 11.05 | 7.51 | 0.28358** | -0.12377 | 0.35219** | 1.00000 |

Note: M and SD indicate mean and standard deviation, respectively. Correlation values represent values of r. P values are denoted by asterisks where * = $p < 0.05$; ** = $p < 0.01$. NDVI = normalized difference vegetative index

Table S2. Means, standard deviations, and Pearson correlations for chapter 2 data.

| **Variable** | **M** | **SD** | **NDVI** | **Bleaching** | **Necrosis** | **Clipping** |
|---|---|---|---|---|---|---|
| **NDVI** | 0.63 | 0.09 | 1.00000 | | | |
| **Bleaching** | 9.36 | 18.83 | -0.24818** | 1.00000 | | |
| **Necrosis** | 11.73 | 19.72 | -0.76936** | 0.07263 | 1.00000 | |
| **Clipping** | 13.80 | 11.21 | 0.04819 | -0.18292* | -0.01493 | 1.00000 |

Note: M and SD indicate mean and standard deviation, respectively. Correlation values represent values of r. P values are denoted by asterisks where * = $p < 0.05$; ** = $p < 0.01$. NDVI = normalized difference vegetative index

Table S3. Means, standard deviations, and Pearson correlations for Chapter 3 data.

| **Variable** | **M** | **SD** | **NDVI** | **Bleaching** | **Necrosis** |
|---|---|---|---|---|---|
| **NDVI** | 0.55 | 0.12 | 1.00000 | | |
| **Bleaching** | 18.05 | 23.40 | -0.85704** | 1.00000 | |
| **Necrosis** | 7.33 | 16.63 | 0.08153 | -0.23067** | 1.00000 |

Note: M and SD indicate mean and standard deviation, respectively. Correlation values represent values of r. P values are denoted by asterisks where * = $p < 0.05$; ** = $p < 0.01$. NDVI = normalized difference vegetative index

Table S4. Means, standard deviations, and Pearson correlations for Chapter 4 data.

| Variable | M | SD | Bleaching | Clipping | Photosynthesis analysis | Chl *a* | Chl *b* | Carotenoids |
|---|---|---|---|---|---|---|---|---|
| **Bleaching** | 4.27 | 10.55 | 1.00000 | | | | | |
| **Clipping** | 0.88 | 0.41 | -0.22795** | 1.00000 | | | | |
| **Photosynthesis analysis** | 10.86 | 6.91 | 0.19238** | -0.35616** | 1.00000 | | | |
| **Chl *a*** | 9.38 | 2.57 | -0.42547** | 0.20535** | -0.19580** | 1.00000 | | |
| **Chl *b*** | 5.28 | 1.95 | -0.21847** | 0.05482 | -0.06000 | 0.56308** | 1.00000 | |
| **Carotenoids** | 1.94 | 0.72 | -0.30100** | 0.20190** | -0.08153 | 0.63854** | 0.03867 | 1.00000 |

Note: M and SD indicate mean and standard deviation, respectively. Correlation values represent values of r. P values are denoted by asterisks where * = $p < 0.05$; ** = $p < 0.01$. Chl *a* = chlorophyll *a*; Chl *b* = chlorophyll *b*