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To the Graduate Council:

I am submitting herewith a dissertation written by Matthew Thomas Elmore entitled "Alternative Strategies for Weed Control in Creeping Bentgrass." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils, and Insects.

James T. Brosnan, Major Professor

We have read this dissertation and recommend its acceptance:

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Alternative Strategies for Weed Control in Creeping Bentgrass

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Matthew Thomas Elmore

August 2014

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ABSTRACT

Creeping bentgrass (CBG) (*Agrostis stolonifera* L.) is the most widely used cool-season turfgrass species on golf course fairways and tees in the United States, but it is tolerant of few post-emergence herbicides. Commercial herbicide development is currently focused on providing superintendents with herbicides that pose minimal environmental impact and can be used effectively at lower application rates than older alternatives. However, few of these products are safe for use on CBG. Several projects were conducted to evaluate alternative strategies to increase CBG herbicide tolerance.

Plants naturally contain enzymatic systems that metabolize xenobiotics. The interspecies variability in these enzymes is exploited by selective herbicides that are rapidly metabolized by desirable plants but not target weeds. Herbicide safeners can further exploit these differences and increase herbicidal selectivity in corn and wheat, but they have not been investigated in turfgrass.

In one project, glasshouse research investigated the efficacy of herbicide safeners for improving CBG tolerance to amicarbazone, bispyribac-sodium, fenoxaprop-p-ethyl, imazapic, quinclorac or topramezone. The herbicide safener cloquintocet-mexyl reduced CBG injury from topramezone and did not affect topramezone efficacy against large crabgrass or goosegrass.

Cytochrome P450 monooxygenases (P450's) are an enzyme superfamily often responsible for metabolizing xenobiotics. An experiment evaluated CBG in hydroponic culture to determine if P450's influence CBG tolerance to topramezone. Known P450 inhibitors increased CBG injury caused by topramezone, suggesting creeping bentgrass tolerance to topramezone is influenced by cytochrome P450-catalyzed metabolism.

Another project investigated safeners in combination with the herbicide pinoxaden. Glasshouse experiments determined that the safeners cloquintocet-mexyl, fenchlorazole-ethyl,

and mefenpyr-diethyl reduced CBG injury in a rate-dependent manner. The safeners did not affect perennial ryegrass control, but reduced roughstalk bluegrass control. However, these reductions were offset by CBG injury reductions.

Another project investigated novel triketone *p*-HPPD inhibitors for their safety to CBG and herbicidal efficacy against goosegrass and crabgrass. Previous research suggests that 2-benzoyl-1,3-cyclohexanedione compounds with alkyl substitutions on the cyclohexane moiety have more efficacy against grassy weeds. We synthesized 2-(2,4-dichlorobenzoyl)cyclohexane-1,3-dione compounds with various alkyl substituents on the cyclohexanedione moiety and evaluated their efficacy against turfgrass weeds. The 5,5-dimethyl-substituted compound had efficacy against goosegrass and did not cause injury to CBG.

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INTRODUCTION

BACKGROUND

Creeping bentgrass (CBG) (*Agrostis stolonifera* L.) is the most widely used cool-season turfgrass species on golf course fairways and tees in the United States (Lyman et al. 2007). Despite widespread popularity, CBG is tolerant of few post-emergence herbicides. This forces turfgrass managers to tolerate moderate injury from herbicides used to control graminaceous weeds or use more laborious methods of removal (Dernoeden et al. 2003; Henry and Hart 2004; Johnson 1994; McDonald et al. 2006). Herbicide development is currently focused on providing superintendents with herbicides that pose minimal environmental impact and can be used effectively at lower application rates than older alternatives; examples include amicarbazone, bispyribac-sodium and topramezone. However, few of these products are safe for use on CBG.

The objective of this research was to explore two strategies to develop herbicide options for weed control in CBG. One strategy is to use herbicide safeners to increase CBG tolerance to herbicides. The other is to synthesize analogs of commercially available herbicides and evaluate their efficacy against turfgrass weeds.

HERBICIDE SAFENERS

Turfgrasses naturally contain enzymatic systems to protect against xenobiotics (synthetic chemicals within the plant). Enzymes responsible for xenobiotic detoxification vary greatly across plant species (Kreuz et al. 1996). This variability is exploited by selective herbicides that are easily detoxified by desirable species but not target weeds. For example, the herbicide bispyribac-sodium is more rapidly detoxified by CBG than annual bluegrass (*Poa annua* L.), leading to selective control of annual bluegrass in CBG (McCullough et al. 2009). However, CBG injury from bispyribac-sodium still occurs, forcing superintendents to either reduce bispyribac-sodium application rates to levels that reduce weed control or tolerate moderate CBG

injury (Dernoeden et al. 2008; Rutledge et al. 2010). Selectively enhancing the ability of CBG to more rapidly detoxify bispyribac-sodium or other herbicides would provide more versatile options for selective weed control.

Ideally, herbicide safeners enhance xenobiotic detoxification in desirable plants but not weeds. The cytochrome P₄₅₀ and glutathione transferase (GST) enzyme families are responsible for detoxifying many herbicides; herbicide safeners increase production of these enzymes in select species leading to more rapid herbicide detoxification and less injury (Edwards et al. 2000; Werck-Reichart et al. 2000). Introduced in 1971, naphthalic anhydride (NA) was the first compound patented exclusively as a herbicide safener (Davies 2001). Since the inception of NA other safeners have been developed. For example, the safener mefenpyr-diethyl reduces injury from the herbicide fenoxaprop-p-ethyl to cereal crops (Hacker et al. 2000). In crop production, other safeners such as cloquintocet-mexyl, isoxadifen-ethyl, furilazole, and cyprosulfamide reduce injury from several different herbicidal classes (Davies 2001; Hatzios 1997; Maldiza et al. 2010). Research investigating the use of safeners in turfgrass found that the pyridine carboxylic acid herbicide, triclopyr, reduces zoysiagrass (*Zoysia japonica* Steud.) injury with fluazifop-p-butyl (Lewis et al. 2010). Other research investigating tall fescue [*Festuca arundinacea* (Scop.) Holub] as a buffer in row crops demonstrated several safeners increased tall fescue metabolism of the herbicides terbuthylazine and butachlor (Scarponi and Del Buono 2009).

Information regarding the use of safeners in CBG weed management programs is needed. Findings could allow superintendents to develop weed management programs centered upon the use of herbicides not currently safe for use on CBG, many of which offer more favorable environmental toxicology and lower use-rates than current options. For example, ethofumesate is applied at rates as high as 900 g ha⁻¹ despite the potential for CBG injury and inconsistent

annual bluegrass control (Branham and Meyer 2006; Kohler and Branham 2002). Herbicides that can be used at lower use rates and offer a more favorable environmental profile (e.g., bispyribac-sodium, amicarbazone, etc.) have been developed for annual bluegrass control in CBG.

However, they are often injurious to CBG, making these herbicides ideal candidates for improvement with safeners (McCullough et al. 2009; McCullough et al. 2010). The same is true for herbicides used to control weed species other than annual bluegrass including imazapic, topramezone and fenoxaprop-p-ethyl.

In recent years, herbicide development has focused on providing superintendents herbicides with minimal environmental impact that can be used effectively at lower application rates than older alternatives. Few of these products are safe for use on CBG. The objective of this research is to determine if safeners can increase CBG tolerance to these new herbicides and maintain weed control efficacy.

TRIKETONE HERBICIDES

Mesotrione is the only HPPD-inhibiting, triketone herbicide registered for use in turfgrass and corn. It is registered for postemergence application at up to 150 g ha⁻¹ in *Zea mays* and the turfgrass *Lolium perenne*, and up to 280 g ha⁻¹ in *Festuca arundinacea* and *Poa pratensis* (Anonymous 2011, 2012). In *Zea mays*, mesotrione is primarily used to control broadleaf weeds. It can provide control of *Digitaria* and *Echinochloa* species at higher application rates, but it is often applied in combination with an ALS-inhibiting herbicide to provide annual grassy weed control (Mitchell et al. 2001; Soltani et al. 2012). In turfgrass, many other effective options for broadleaf weed control are available, therefore, mesotrione is used primarily for selective control of grassy weeds such as *Agrostis stolonifera*, *Poa annua*, and *Digitaria* species. However, complete control of these grassy weeds often requires multiple applications (Elmore et al. 2012;

Jones and Christians 2007; Skelton et al. 2012). The efficacy of triketones with alkyl-substituted 1,3-cyclohexanedione moieties against graminaceous weeds problematic in turfgrass has not been evaluated. HPPD-inhibitors with more efficacy and selectivity for certain weedy grasses would have utility in turfgrass management.

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**CHAPTER 1: HERBICIDE SAFENERS INCREASE CREEPING
BENTGRASS TOLERANCE TO TOPRAMEZONE**

This chapter is based on a paper submitted for publication on April 23, 2014 by Matthew Elmore, James Brosnan, Gregory Armel, Javier Vargas and Gregory Breeden:

Elmore, M.T., J.T. Brosnan, G.R. Armel, J.J. Vargas and G.K. Breeden. 2014. Herbicide Safeners Increase Creeping Bentgrass (*Agrostis stolonifera*) Tolerance to Topramezone. Weed Technology. (*Under review*).

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

ABSTRACT

Glasshouse research was conducted to investigate the efficacy of herbicide safeners for improving creeping bentgrass (CBG) tolerance to various herbicides. CBG injury from amicarbazone (150 g ha⁻¹), bispyribac-sodium (110 g ha⁻¹) fenoxaprop-p-ethyl (35 g ha⁻¹), imazapic (45 g ha⁻¹), quinclorac (1050 g ha⁻¹) or topramezone (37 g ha⁻¹) applied in combination with the herbicide safeners naphthalic anhydride or isoxadifen-ethyl was evaluated. These safeners reduced CBG injury from topramezone. Topramezone was then applied in combination with naphthalic anhydride, isoxadifen-ethyl, cloquintocet-mexyl (cloquintocet), fenchlorazole-ethyl, mefenpyr-diethyl and benoxacor. These experiments determined that CBG injury was lowest from topramezone in combination with cloquintocet. Additional experiments evaluated topramezone (37 g ha⁻¹) with several rates of cloquintocet and determined that applications at \geq 28 g ha⁻¹ reduced CBG injury similarly. Cloquintocet (28 g ha⁻¹) increased topramezone I₅₀ values against CBG, but not large crabgrass or goosegrass. The cytochrome P450 (cP450)

inhibitor malathion (1000 g ha^{-1}) reduced topramezone I_{50} values against CBG in one experimental run. Topramezone-cloquintocet combinations warrant further research in field settings.

INTRODUCTION

Creeping bentgrass (CBG) is the most widely used cool-season turfgrass on golf course fairways and tees in the United States (Lyman et al. 2007). Despite widespread usage, there are few efficacious options for POST control of warm-season graminaceous weeds such as crabgrass (*Digitaria* spp.), goosegrass and bermudagrass (*Cynodon* spp.), as herbicide rates required to provide acceptable weed control are too injurious to desirable CBG.

Desirable herbicide safeners protect graminaceous crop plants from herbicide injury more than target weeds, increasing the margin of selectivity. Safeners function predominately by increasing activity of proteins that catalyze Phase I or II reactions involved in herbicide metabolism. Hydroxylation, deamination, oxidation and N-dealkylation are Phase I reactions catalyzed by cP450 monooxygenase enzymes. These reactions can predispose the herbicide molecule to Phase II reactions that involve conjugation with glutathione, glucose or amino acids. These Phase II reactions can be catalyzed by enzymes such as glutathione s-transferases, glucosyl transferases and UDP-glucose transferases (Chapple 1998; Davies et al. 1998; Hatzios and Burgos 2004; Kreuz et al. 1996).

Safener selectivity is dependent on either application placement or different herbicide-safener interactions among crops and weeds (Hatzios and Burgos 2004). For example, the broad-spectrum safener naphthalic anhydride was applied to corn (*Zea mays* L.) seed to prevent injury from thiocarbamate herbicides and has been shown experimentally to safen corn, and other graminaceous crops to a wide range of herbicides (Davies et. al 2001; Parker 1983). Advances in safener technology have yielded safeners such as isoxadifen-ethyl and cyprosulfamide that are sold commercially to increase the tolerance of corn to herbicides such as isoxaflutole, foramsulfuron, thiencazone-methyl. Other safeners such as cloquintocet, fenchlorazole-ethyl

and mefenpyr-diethyl are used to increase the tolerance of cereal crops to aryloxyphenoxypropionate (AOPP) and sulfonyleurea herbicides (Davies and Cassely 1999; Hatzios and Burgos 2004). Safeners used in cereal crops are applied in pre-packaged mixtures with the herbicide at safener:herbicide ratios typically ranging from 1:4 to 1:6 (Hatzios and Burgos 2004). Safeners such as isoxadifen-ethyl, fenchlorazole-ethyl, mefenpyr-diethyl and cloquintocet are absorbed through the foliage and de-esterified, resulting in a free acid that is thought to be the active agent (Taylor et al. 2013).

Use of several herbicides is limited or prohibited on CBG because they cause too much turfgrass injury at rates that provide acceptable weed control. However, these herbicides are registered for use in other cool-season turfgrass species, which suggests effective safeners may have utility in CBG management. There are no reports of increased CBG tolerance to herbicides after application of compounds used exclusively as safeners. However, other researchers have reported that triclopyr, a pyrimidine carboxylic acid herbicide, can increase zoysiagrass (*Zoysia japonica* Steud.) tolerance to AOPP herbicides (Doroh et al. 2010; Lewis et al. 2010; McElroy and Breeden 2006).

The AOPP herbicide fenoxaprop-*p*-ethyl is widely used to control crabgrass and goosegrass in cool-season turfgrass at 200 g ha⁻¹ (Neal et al. 1990). However, fenoxaprop-*p*-ethyl can cause phytotoxicity to CBG when applied at > 40 g ha⁻¹ (Dernoeden 1987; Henry and Hart 2004; Shim and Johnson 1992). Current fenoxaprop-*p*-ethyl labeling prohibits applications at > 18 g ha⁻¹ to CBG fairways and prohibits use on putting greens altogether (Anonymous 2004). These reduced rates make controlling crabgrass and goosegrass infestations in CBG challenging. Dernoeden et al. (1992) reported multiple applications of fenoxaprop-ethyl at 36 g ha⁻¹ were required to provide acceptable control of 1 to 2 leaf smooth crabgrass [*Digitaria ischaemum*

(Schreb.) Schreb. ex Muhl] in a perennial ryegrass (*Lolium perenne* L.) fairway. Improved fenoxaprop-*p*-ethyl utility would require greater CBG tolerance to permit applications at $>18 \text{ g ha}^{-1}$. More rapid metabolism of the herbicidally active fenoxaprop acid to inactive metabolites in wheat (*Triticum* spp.), compared to weed species such as smooth crabgrass allows for fenoxaprop-*p*-ethyl utility in wheat. The safener fenchlorazole-ethyl can increase the rate of fenoxaprop-*p*-ethyl metabolism in wheat without doing the same in weeds, improving selectivity. (Lefsrund and Hall 1989; Stephenson et al. 1993; Tal et al. 1995; Yaacoby et al. 1991).

Quinclorac can be applied to cool-season turfgrasses such as tall fescue (*Festuca arundinacea* Schreb.), Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass at rates up to 840 g ha^{-1} (Anonymous 2012a). However, quinclorac is labeled for use on CBG fairways at lower rates ($280 \text{ to } 560 \text{ g ha}^{-1}$) (Anonymous 2012a; Chism and Bingham 1991; Dernoeden et al. 2003; Zawierucha and Penner 2001). Even at these reduced rates, quinclorac applications often result in CBG leaf chlorosis that can lead to stand density reductions, especially if applications are made prior to or during high temperatures (Dernoeden et al. 2003; Hart et al. 2004; Johnson 1994; Reicher et al. 2002).

Amicarbazone is a photosystem II-inhibiting herbicide registered for application on CBG fairways at $\leq 50 \text{ g ha}^{-1}$ for selective annual bluegrass control (Anonymous 2012b; Dayan et al. 2009). Amicarbazone injury has been reported in fairway-height CBG following sequential fall applications at 100 g ha^{-1} (McCullough et al. 2010). Other researchers have reported CBG injury at rates as low as 49 g ha^{-1} on putting greens and in glasshouse and growth chamber experiments (Brosnan et al. 2013; Jefferies et al. 2012). The selectivity of amicarbazone for annual bluegrass in CBG is at least partially attributed to metabolism (Yu et al. 2013).

Topramezone is a p-hydroxyphenylpyruvate dioxygenase (HPPD; EC 1.13.11.27)-inhibiting herbicide registered for use in Kentucky bluegrass, tall fescue, fine fescue (*Festuca* spp.), perennial ryegrass and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] at ≤ 37 g ha⁻¹ (Anonymous 2013, Grossman and Ehrhardt 2007). When applied between 12 and 37 g ha⁻¹, topramezone can control multi-tiller crabgrass and goosegrass, while sequential applications can suppress bermudagrass (Brosnan and Breeden 2013; Elmore et al. 2012; Smith et al. 2013). However, topramezone can cause injury to CBG when applied at 6 to 37 g ha⁻¹ and therefore is not registered for use in CBG (Smith et al. 2013; Venner et al. 2014). Rapid N-demethylation of topramezone to an inactive metabolite has been attributed to excellent corn tolerance (Grossman and Ehrhardt 2007).

Bispyribac-sodium is an acetolactate synthase (ALS; EC 2.2.16)-inhibiting herbicide labeled for use on CBG fairways at up to 74 g ha⁻¹ (Anonymous 2010; Shimizu 1997). However, lack of selectivity for ABG in CBG causes transient injury, which can make bispyribac-sodium applications problematic, especially in cooler temperatures in the fall (McDonald et al. 2006; Park et al. 2002). The selectivity of bispyribac-sodium for annual bluegrass in CBG is at least partially attributed to metabolism (McCullough et al. 2009).

Imidazolinone herbicides such as imazapic have been evaluated for growth regulation and seedhead suppression in warm- and cool-season turfgrasses (Brosnan et al. 2011, 2012; Baker et al. 1999; Gannon and Yelverton 2011; Hixon et al. 2007; McCarty et al. 2004). Imazapic can control large crabgrass and other weeds, but there are no reports describing CBG tolerance (Jordan et al. 2009). Preliminary research conducted at the University of Tennessee demonstrated transient 'Penncross' CBG injury from imazapic (65 g ha⁻¹). The safener

naphthalic anhydride can increase cP450-mediated hydroxylation of imazapic in maize (Davies et al. 1998). A similar effect in CBG may give imazapic utility in CBG weed management.

Since there are no reports of safener efficacy in turfgrass, the objective of this research was to evaluate CBG tolerance to various herbicides in combination with herbicide safeners. Additionally, our objective was to determine the effect of various safener rates on CBG tolerance and graminaceous weed control. These herbicides comprise five different sites of action and six different chemical families. Increasing CBG tolerance to these herbicides would increase weed control options in CBG. Our hypothesis was that CBG tolerance to some herbicides evaluated will be improved but safener rates of specific herbicide-safener combinations will require optimization.

MATERIALS AND METHODS

Plant Culture. Glasshouse experiments were conducted during 2012 and 2013 in Knoxville, TN (35° 57' N lat.) under ambient light. 'Penncross' CBG was seeded to 6.5-cm diameter cone-tainers (Stuewe and Sons, Tangent, OR) filled with a peat moss, perlite and vermiculite growing medium (Fafard No. 2, Sun Gro Horticulture, Agawam, MA) and maintained in a glasshouse at a 1.3 cm height using mechanical shears (Showmaster, Oster Professional Products, McMinnville, TN) for six months before treatment. During this time, CBG was fertilized on an as-needed basis using a complete (20N:20P₂O₅:20K₂O) fertilizer (Howard Johnson's Triple Twenty Plus Minors, Milwaukee, WI) at 25 kg N ha⁻¹ and irrigated as needed to prevent wilt. Beginning 10 days prior to treatment application, CBG was fertilized with a complete fertilizer at 10 kg N ha⁻¹ every 10 days for the duration of the experiment. Plants were hand-trimmed to a 1.3-cm height with

scissors approximately 24 h before treatment and mowing was then suspended for the duration of experimentation.

Experiment 1: Identify Candidate Herbicides. Research was conducted to determine which herbicides could be candidates for use on CBG when combined with a herbicide safener. All treatments were compared to a non-treated control. Herbicide treatments listed in Table 1 were applied alone or in combination with the herbicide safeners naphthalic anhydride (1,8-naphthalic anhydride; Sigma Aldrich, St. Louis, MO) or isoxadifen-ethyl (50 WDG; DuPont Ag Products, Wilmington, DE). Herbicide rates were based on current product labeling and preliminary field research that determined rates expected to cause 15 to 30% injury. Safeners were applied at 0, 5 or 10 times the rate of each herbicide to maximize potential for safener efficacy. Safeners were also applied at two timings: three days or 30 min before herbicide application. This formed a four-way (herbicide, safener, safener rate, safener timing) factorial treatment design with three replications. Safeners were applied to non-herbicide-treated CBG to ensure they were not phytotoxic.

All treatments except for quinclorac were applied with non-ionic surfactant (Activator 90. Loveland Products Inc., Loveland, CO) at 0.25% v/v. Quinclorac was applied with a methylated seed oil (Loveland Products Inc., Loveland, CO) (MSO) at 1% v/v according to the product label (Anonymous 2012a). All treatments were applied with 215 L ha⁻¹ of water carrier through a single flat-fan nozzle (8004 EVS; Spraying Systems Co., Roswell, GA) in a spray chamber (Generation III Research Track Sprayer. DeVries Manufacturing, Hollandale, MN). Naphthalic anhydride was dissolved in 3 ml of acetone prior to addition to appropriate spray solutions.

Irrigation was withheld for 18 h after treatment application. Herbicides were applied on 7 February and 1 June 2012 for experimental runs A and B, respectively.

CBG injury was evaluated 1, 2 and 3 weeks after treatment (WAT) on a 0 (no injury) to 100% (complete leaf tissue necrosis) scale. Only CBG injury data collected 2 WAT will be presented, as treatment responses were most apparent at this time. Additionally, clipping yield was quantified 3 WAT. Scissors were used to remove foliage above 1.3 cm. Harvested tissue was then placed in a drying oven at 80°C for 48 h and weighed.

Experiment 2: Identify Candidate Safeners. Based on the results of Experiment 1, research was conducted to determine safener candidates for use on CBG when combined with topramezone. CBG was treated with safeners listed in Table 2 at a 5:1 safener:herbicide ratio 30 min prior to application of topramezone (37 g ha⁻¹). Comparisons were made to a non-treated control. Plant culture, treatment application and evaluation were conducted in the same manner as in Experiment 1. Additionally, CBG injury was also quantified via measurements of turfgrass cover via digital image analysis (DIA) using methods modified from Richardson (2001) and Karcher and Richardson (2003). CBG cone-tainers were placed into a custom-constructed light box with a blue background and digitally photographed with a Canon G12 (Canon Inc., Japan) camera. Sigma Scan Pro Software (v. 5.0, SPSS. Inc., Chicago, IL) was used to determine the number of green pixels in each image. Green pixels were defined as those having a hue between 60 and 102 and saturation between 0 and 100 percent to exclude herbicide-injured leaves (defined as hue < 60; 103 to 134) and the blue background (hue range 135 to 153) from selection.

Treatments were arranged in a single factor treatment design with three replications. Herbicides were applied on 16 and 30 August 2012 for experimental runs A and B, respectively.

Experiment 3: Evaluate Safener Efficacy at Reduced Rates. Additional glasshouse research evaluated the efficacy of various safener rates to reduce CBG injury from topramezone with effective herbicide-safener combinations identified in Experiments 1 and 2. Cloquintocet was evaluated at 185, 93, 37, 28, 18, 9 and 0 g ha⁻¹ in combination with topramezone at 37 g ha⁻¹. CBG was treated with cloquintocet 30 min prior to topramezone application. Comparisons were made to a non-treated control. Treatments were arranged in a single-factor, completely randomized design with four replications. Plant culture, treatment application, and data collection were conducted in the same manner as Experiment 2. Treatments for experimental runs A and B were applied on 19 March and 11 October 2013.

Experiment 4: Comparing Responses on CBG and Weeds. Additional research was conducted to compare the response of CBG and graminaceous weeds to herbicide-safener combinations identified in Experiments 1, 2 and 3. CBG was maintained according to previously described methods. Large crabgrass and goosegrass were seeded to separate 10-by-10 cm square pots filled with peat moss, perlite and vermiculite growing medium described previously. During this time, they were fertilized on an as-needed basis using a complete (20N:20P₂O₅:20K₂O) fertilizer (Howard Johnson's Triple Twenty Plus Minors, Milwaukee, WI) at 25 kg N ha⁻¹ and irrigated as needed to prevent wilt. After emergence, pots were hand-thinned to contain 2 plants. Scissors were used to maintain large crabgrass and goosegrass at a 10 cm height prior to

treatment. Herbicide-safener combinations were applied to goosegrass and large crabgrass plants at the three-to-five tiller growth stage.

Large crabgrass, goosegrass and CBG plants were treated with either the safener cloquintocet (28 g ha^{-1}), the cP450-inhibitor malathion (1000 g ha^{-1}), or left non-treated 30 min prior to treatment with topramezone at 0, 2, 4, 8, 16, 32, 64 or 128 g ha^{-1} . Malathion was used to inhibit cP450 function *in vivo* and indirectly evaluate the influence of cP450-catalyzed metabolism on CBG tolerance to topramezone (Yun et al. 2005). Treatments were applied in the same manner as previously described for Experiments 1, 2 and 3. CBG visible injury was evaluated on 0 (i.e., no injury) to 100% (i.e., complete leaf tissue necrosis) scale and using DIA at 1, 2 and 3 WAT. Goosegrass and large crabgrass injury were evaluated at 1, 2 and 3 WAT on a similar 0 to 100% scale. Aboveground biomass data were collected for large crabgrass and goosegrass 3 WAT as described previously. Visible injury data collected 2 WAT will be presented, as treatment responses were greatest at this time. Pots were arranged in a completely randomized design with four replications. Treatments for experimental runs A and B were applied on 2 and 14 October 2013, respectively.

Statistical Analysis. In all experiments, model assumptions were tested through residual analysis (Shapiro–Wilk statistic) in SAS version 9.3 (Statistical Analysis Software, Inc., Cary, NC). Visible injury data in Experiments 1 and 2 were subjected to arcsine-square root transformation prior to analysis. Untransformed data are presented as interpretations were the same. In Experiment 2, t-tests ($\alpha \leq 0.05$) were used to compare the each safener treatments to the herbicide-treated control. In all experiments, ANOVA was conducted in SAS with main effects and all possible interactions tested using the appropriate expected mean square values as

described by McIntosh (1983). Fisher's protected LSD ($\alpha \leq 0.05$) was used to separate means. Pearson's correlation coefficients of visible injury and turfgrass cover as determined by DIA were conducted in SAS ($\alpha \leq 0.05$). In Experiment 4, I_{50} (i.e., the herbicide dose required to cause 50% visible injury) and GR_{50} (i.e., the herbicide dose required to cause a 50% reduction in aboveground biomass) values were calculated using a log-logistic model in Prism (Prism 5.0 for Mac OSX, GraphPad Software, LaJolla, CA):

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

Where Y represents large crabgrass or goosegrass injury, X represents the \log_{10} of the herbicide dose applied, C is the lower limit for Y (i.e., 0% large crabgrass control or goosegrass injury), D is the upper limit for Y (i.e., 100% large crabgrass control or goosegrass injury), I_{50} is the herbicide dose resulting in 50% injury, B is the slope of the line at I_{50} , and (Seefeldt et al. 1995). Significance of main effect interactions of herbicide dose-by-cloquintocet or malathion in Experiment 4 were evaluated using non-linear regression and a lack of fit F-test ($\alpha \leq 0.05$) in Prism as well.

RESULTS AND DISCUSSION

Experiment 1: Identify Candidate Herbicides. Main effect interactions with experimental run were significant; therefore, data from each experimental run were analyzed separately.

The main effect of herbicide was significant in both experimental runs. Averaged across main effects, amicarbazone was the most injurious herbicide to CBG; treated plants displayed 29 and 49% injury in Runs A and B, respectively, 2 WAT. This injury is similar to that reported in growth chamber experiments by McCullough et al. (2010). Topramezone-treated CBG displayed 15 and 9% injury 2 WAT in runs A and B, respectively. All other treatments displayed < 10%

CBG injury 2 WAT. Despite injuring CBG < 10%, clipping yields of imazapic and fenoxaprop-treated plants were reduced 40 to 51% compared to non-herbicide-treated CBG. Henry and Hart (2004) reported similar levels of injury after a single fenoxaprop application at $\leq 40 \text{ g ha}^{-1}$, but only observed < 20% clipping yield reduction on 'L-93' CBG. However, the researchers collected clipping data 4 WAT; anecdotal observations from our experiments suggest CBG shoot growth was mostly reduced for approximately 2 WAT, after which time it began to recover.

A significant herbicide-by-safener interaction was detected in visible CBG data collected in Run A. Topramezone injury was reduced from 26% when no safener was applied to 17 and 11% when naphthalic anhydride and isoxadifen were applied, respectively (data not presented). Similarly, topramezone reduced CBG clipping yield by 28% compared to the non-treated-control; when applied with naphthalic anhydride or isoxadifen, topramezone only reduced clipping yield < 15% in Run A. Herbicide interactions with safener rate or timing were not significant in either experimental run, suggesting no advantage to applying the safener 3 days prior to the herbicide or applying the safener at a 10:1 herbicide-to-safener ratio instead of a 5:1 ratio.

Experiment 2: Identify Candidate Safeners. Safener-by-experimental run interactions were not detected; therefore, data were pooled across experimental runs A and B.

Topramezone caused 23% CBG injury when applied alone. When topramezone was applied following treatment with cloquintocet (185 g ha^{-1}) CBG injury only measured 11%. When topramezone was applied following treatment with other safeners listed in Table 1.2, CBG injury was similar to CBG treated with topramezone alone (data not presented). CBG injury and turfgrass cover were well correlated ($r = -0.77$; $P < 0.0001$) at 2 WAT. Hoyle et al. (2013)

reported similar regression coefficients ($r = 0.78$ to 0.90) between evaluations of percent crabgrass cover and DIA after application of the HPPD-inhibitor mesotrione.

Experiment 3: Evaluate Safener Efficacy at Reduced Rates. Interactions of safener rate with experimental run were not detected; therefore data were pooled across experimental runs.

Similar to observations from Experiment 2, topramezone applied without cloquintocet caused 23% CBG injury 2 WAT (Table 1.3). Cloquintocet application reduced injury to $\leq 11\%$ 2 WAT when applied at rates $\geq 28 \text{ g ha}^{-1}$. Data suggest there is no benefit to applying cloquintocet at $> 28 \text{ g ha}^{-1}$ in combination with topramezone at 37 g ha^{-1} . CBG injury observed in this experiment (as well as Experiments 1 and 2) was less than the 30 to 40% reported by Venner et al. (2014) from topramezone application at 25 g ha^{-1} in early or late summer. CBG injury and turfgrass cover were weakly correlated ($r = -0.54$; $P < 0.0001$) in Experiment 3 at 2 WAT.

Experiment 4: Dose-Response Experiments. Main effect interactions with experimental run were significant; therefore, data from each experimental run were analyzed separately. CBG injury and turfgrass cover were well correlated in Run A ($r = -0.79$; $P < 0.0001$) and B ($r = -0.92$; $P < 0.0001$).

In both experimental runs, the main effect of topramezone rate significantly affected injury and aboveground biomass data collected on goosegrass and large crabgrass; the effect of topramezone rate was also significant for CBG injury and turfgrass cover at 2 WAT. The main effect of cloquintocet and malathion was not significant crabgrass or goosegrass control 2 WAT in Run A. Cloquintocet and malathion did affect topramezone injury to CBG.

Treatment with cloquintocet and malathion affected topramezone I_{50} values against CBG. In Run A, the I_{50} value for topramezone applied alone was 60 g ha^{-1} (Figure 1). Treatment with cloquintocet increased the topramezone I_{50} value against CBG to 114 g ha^{-1} while malathion reduced it to 42 g ha^{-1} . In Run B, the I_{50} value for topramezone applied alone was 32 g ha^{-1} . Cloquintocet increased the I_{50} value to 52 g ha^{-1} while malathion had no effect on the I_{50} value for topramezone in Run B. These data indicate that cloquintocet (28 g ha^{-1}) reduces CBG injury caused by topramezone application across several herbicide rates. Malathion increased CBG injury caused by topramezone in one experimental run, suggesting cP450-catalyzed metabolism may influence CBG tolerance to topramezone. However, additional research is needed to explore this concept in further detail. Even in combination with the cP450 inhibitor malathion, I_{50} values for topramezone were higher in CBG ($\leq 42 \text{ g ha}^{-1}$) than when topramezone was applied alone to goosegrass or large crabgrass ($< 10 \text{ g ha}^{-1}$) suggesting that CBG tolerance to topramezone may be conferred by additional mechanisms.

Cloquintocet and malathion generally has less effect on topramezone efficacy against large crabgrass and goosegrass than CBG. Regression analyses determined that large crabgrass and goosegrass I_{50} and GR_{50} values were not affected by the inclusion of cloquintocet or malathion in Run A. Topramezone I_{50} and GR_{50} values against large crabgrass were 5.5 and 8.3 g ha^{-1} in Run A, respectively; I_{50} and GR_{50} values against goosegrass were 1.3 and 6.1 g ha^{-1} , respectively (data not presented). In Run B, compared to topramezone alone, cloquintocet and malathion reduced topramezone GR_{50} values against large crabgrass from 4.3 to $\leq 2.3 \text{ g ha}^{-1}$; goosegrass GR_{50} values were reduced from 2.6 to 1.7 g ha^{-1} . Unlike GR_{50} values, topramezone I_{50} values against crabgrass and goosegrass (11.1 and 10.1 g ha^{-1} , respectively) were not affected by

cloquintocet or malathion in Run B. These data indicate that cloquintocet does not reduce efficacy of topramezone against large crabgrass and goosegrass.

Our findings suggest that cP450-catalyzed metabolism may influence CBG tolerance to topramezone. However, there may be additional mechanisms responsible for this response including a reduced affinity of topramezone for CBG HPPD relative to target weeds; this has been reported as one mechanism of maize tolerance to topramezone (Grossman and Ehrhardt 2007). It is also possible that significant topramezone metabolism may occur via conjugation reactions that do not require cP450-mediated topramezone N-demethylation. Cloquintocet has been reported to increase pyridinyl N-glucosylation of clodinafop-propargyl in wheat (Kreuz et al. 1991). Topramezone N-glycosylation via N-glucosyltransferase at the un-saturated pyrimidinyl N could occur without prior topramezone metabolism. Metabolism may also proceed via O-glucosylation at hydroxyl groups; cloquintocet can increase O-glycosyltransferase activity in wheat (*Triticum aestivum* L.) (Brazier et al. 2002). Future research should explore CBG metabolism of topramezone in greater detail.

Our glasshouse data indicate that topramezone-cloquintocet combinations warrant further research under field conditions. Other researchers have reported that CBG tolerance to topramezone varies seasonally (Venner et al. 2014). Field research should evaluate CBG injury and graminaceous weed control from applications of topramezone in combination with cloquintocet at different seasonal application timings.

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APPENDIX
TABLES AND FIGURES

Table 1.1. Herbicides applied in Experiment 1 to ‘Penncross’ creeping bentgrass in a glasshouse in Knoxville, TN in 2012. Herbicide rates were based on current product labeling and preliminary field research that determined rates expected to cause 15 to 30% injury.

Common Name	Trade Name	Formulation	Rate (g ha ⁻¹)	Manufacturer
Amicarbazone ^a	Xonerate	75 WDG	150	Arysta Lifescience North America LLC; Cary, NC
Bispyribac-sodium	Velocity	17.6 SG	110	Valent Professional Products; Walnut Creek, CA
Fenoxaprop-ethyl	Acclaim Extra	0.57 EC	35	Bayer Environmental Science; Research Triangle Park, NC
Imazapic	Plateau	2 SL	45	BASF Corporation; Research Triangle Park, NC
Quinclorac	Drive XLR8	1.5 SL	1050	BASF Corporation; Research Triangle Park, NC
Topramezone	Pylex	2.8 SC	37	BASF Corporation; Research Triangle Park, NC

^aAll herbicides except for quinclorac were applied with non-ionic surfactant at 0.25% v/v. Quinclorac was applied with methylated seed oil at 1.0% v/v.

Table 1.2. Safeners applied in Experiment 2 to ‘Penncross’ creeping bentgrass in a glasshouse in Knoxville, TN in 2013. Safeners were applied at 185 g ha⁻¹ 30 min prior to treatment with topramezone at 37 g ha⁻¹.

Common name	Trade		Manufacturer
	name	Formulation	
Naphthalic anhydride ^a	N/A	> 97% Tech.	Acros Organics; Pittsburgh, PA
Isoxadifen-ethyl	N/A	50 WDG	DuPont Ag Products; Wilmington, DE
Benoxacor	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC
Cloquintocet-mexyl	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC
Fenchlorazole-ethyl	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC
Mefenpyr-diethyl	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC

^aSafeners were applied with non-ionic surfactant at 0.25% v/v.

Table 1.3. Creeping bentgrass injury and percent green cover as determined by digital image analysis (DIA) 2 WAT with topramezone (37 g ha⁻¹) in combination with cloquintocet-mexyl at 0, 9, 19, 28, 37, 93 or 185 g ha⁻¹. Treatments were applied on 1 and 14 October 2013 for experimental runs A and B, respectively. Data were combined across experimental runs.

Cloquintocet-mexyl ^a	Herbicide:safener	2 WAT ^b		% Green cover	
g ha ⁻¹		—%—		—% chk ^d —	
0	0	23	ab ^c	87	a
9	4:1	25	a	89	a
19	2:1	15	bc	99	a
28	4:3	11	dc	95	a
37	1:1	10	dc	99	a
93	1:2.5	9	dc	93	a
185	1:5	7	d	94	a

^a Cloquintocet-mexyl was applied < 20 min prior to topramezone application. Topramezone and cloquintocet-mexyl were applied with NIS (0.25% v/v).

^b Abbreviations: Weeks after treatment, WAT.

^c Means followed by the same letter are not significantly different as determined by Fisher's protected least significant difference test ($\alpha \leq 0.05$).

^d Data from DIA were transformed to a percentage of the non-treated control.

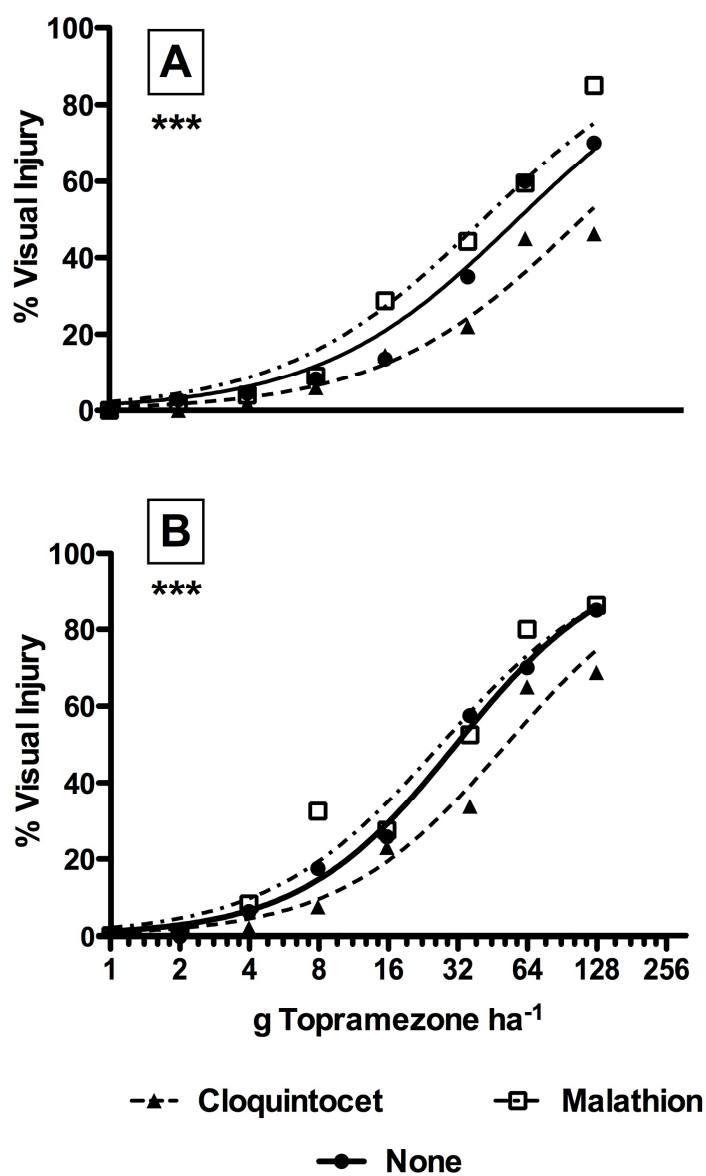


Figure 1.1. Creeping bentgrass injury 2 weeks after treatment with topramezone at 0, 2, 4, 8, 16, 32, 64 or 128 g ha⁻¹ alone or in combination with cloquintocet-mexyl (28 g ha⁻¹) or malathion (1000 g ha⁻¹). *** indicates regression curves were not similar ($\alpha \leq 0.001$). Data from experimental runs A and B are presented separately.

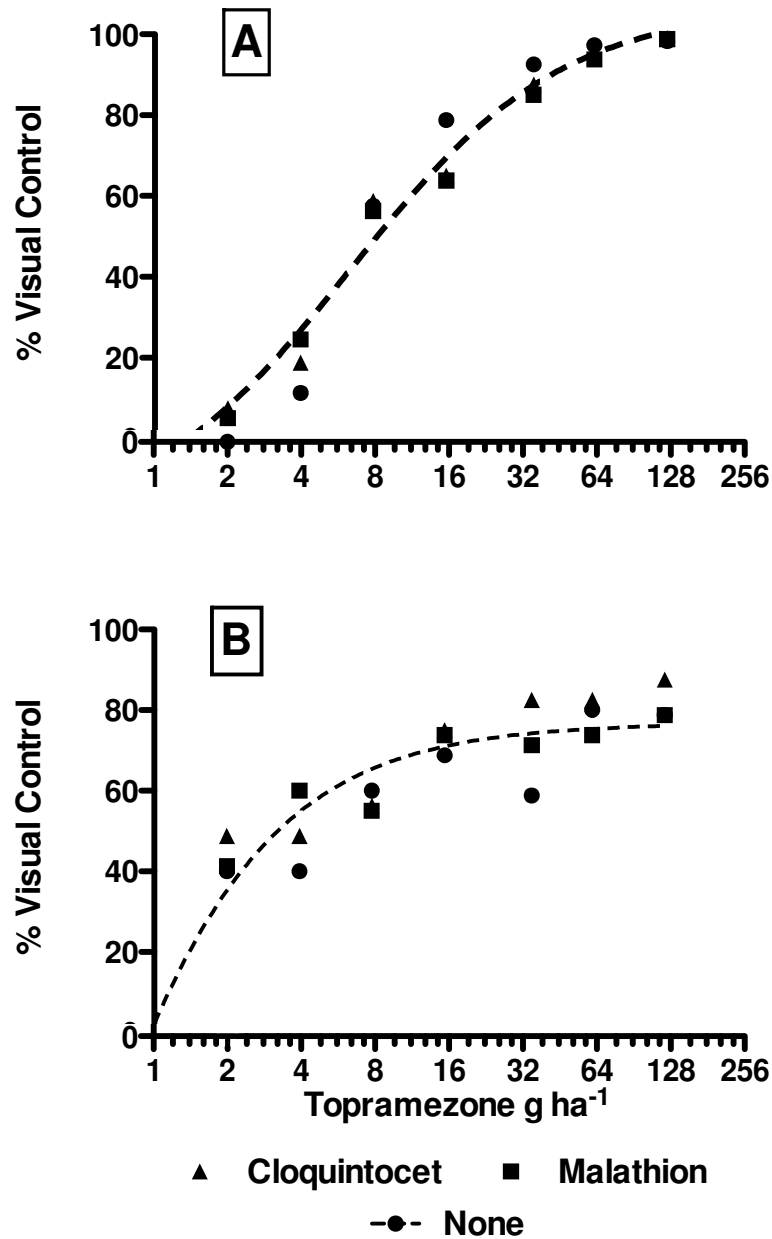


Figure 1.2. Large crabgrass (A) and goosegrass (B) control 2 weeks after treatment with topramezone at 0, 2, 4, 8, 16, 32, 64 or 128 g ha⁻¹ alone or in combination with cloquintocet-mexyl (28 g ha⁻¹) or malathion (1000 g ha⁻¹) in experimental run A. Regression curves were not different ($\alpha \leq 0.05$) in the presence of cloquintocet-mexyl or malathion; therefore data were combined to form one regression curve.

**CHAPTER 2: HERBICIDE SAFENERS INCREASE CREEPING
BENTGRASS TOLERANCE TO PINOXADEN AND AFFECT WEED
CONTROL**

This chapter is based on a paper that will be submitted for publication by Matthew Elmore, James Brosnan, Gregory Armel, Javier Vargas and Gregory Breeden:

Elmore, M.T., J.T. Brosnan, G.R. Armel, J.J. Vargas and G.K. Breeden. 2014. Herbicide Safeners Increase Creeping Bentgrass (*Agrostis stolonifera*) Tolerance to Pinoxaden and Affect Weed Control. Weed Technology.

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

ABSTRACT

The herbicide pinoxaden is a phenylpyrazoline inhibitor of acetyl-CoA carboxylase. Research was conducted to determine the effects of pinoxaden (90 g ha^{-1}) alone and in combination with herbicide safeners on creeping bentgrass injury and perennial ryegrass and roughstalk bluegrass control. Greenhouse experiments determined that cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were more effective in reducing creeping bentgrass injury than benoxacor, isoxadifen-ethyl and naphthalic-anhydride. Other experiments determined that creeping bentgrass injury from pinoxaden decreased as rates (0, 23, 45, 68, 90, 225, or 450 g ha^{-1}) of cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl increased. Based on creeping bentgrass responses to various safener rates, safeners were applied at 68 and 450 g ha^{-1} to evaluate their effects on pinoxaden (90 g ha^{-1}) injury to creeping bentgrass and efficacy against perennial ryegrass and roughstalk bluegrass. While safeners reduced roughstalk bluegrass control

these materials also reduced creeping bentgrass injury from 25% to $\leq 5\%$. Safeners did not affect perennial ryegrass control. Field experiments should evaluate pinoxaden in combination with cloquintocet-mexyl and mefenpyr-diethyl to optimize safener:herbicide ratios and rates for creeping bentgrass safety as well as perennial ryegrass and roughstalk bluegrass control in different climates and seasons.

INTRODUCTION

Creeping bentgrass (CBG) is the most widely used cool-season turfgrass on golf courses in the United States (Lyman et al. 2007). Perennial ryegrass and roughstalk bluegrass are problematic and difficult to control weeds of CBG turf (Turgeon et al. 2009). Roughstalk bluegrass disrupts the functional quality of CBG turf, especially as it goes dormant in the summer (Dernoeden 2013; Turgeon et al. 2009). Perennial ryegrass disrupts CBG stand uniformity with a bunch-type growth and dark green color (Dernoeden 2013; Turgeon et al. 2009).

The acetolactate synthase (ALS) inhibiting herbicides sulfosulfuron and bispyribac-sodium have efficacy for roughstalk bluegrass control in CBG. Sequential applications of sulfosulfuron (13 to 27 g ha⁻¹) and bispyribac-sodium (56 to 74 g ha⁻¹) can control roughstalk bluegrass in combination with CBG interseeding (Rutledge et al. 2010). Other researchers have reported three and four sequential applications, respectively, of bispyribac-sodium (56 to 74 g ha⁻¹) and sulfosulfuron (27 g ha⁻¹) reduce roughstalk bluegrass cover in CBG when temperatures exceed 21°C (Morton et al. 2007). Lycan and Hart (2004) reported sulfosulfuron caused stunting and chlorosis of perennial ryegrass at > 22 g ha⁻¹, but complete recovery was evident 8 WAT; they suggested sequential applications may be required to provide control. McCullough et al. (2008) demonstrated CBG tolerance to sulfosulfuron application at 22 g ha⁻¹ is similar to bispyribac-sodium at 74 g ha⁻¹. However, since sulfosulfuron is not labeled for use in CBG, bispyribac-sodium is the only herbicide registered for roughstalk bluegrass control in CBG (Anonymous 2010a; Anonymous 2012a). The ALS-inhibiting herbicide chlorsulfuron is labeled for perennial ryegrass control in CBG but it must be applied during early autumn to avoid CBG injury (Anonymous 2005; Dernoeden et al. 2013).

Pinoxaden is registered in turfgrass as Rescue® for use in the United Kingdom to control ryegrass (*Lolium* spp.) in fine-leaf fescue (*Festuca* spp.) and annual bluegrass (*Poa annua* L.) at up to 60 g ha⁻¹ (Anonymous 2010b). Pinoxaden is a phenylpyrazoline herbicide that inhibits acetyl-coenzyme A carboxylase (ACCase) in susceptible plants. It was introduced in 2006 to control certain ACCase and ALS-resistant ryegrass (*Lolium* spp.) populations in wheat (*Triticum* spp.) and barley (*Hordeum* spp.) (Muehlebach et al. 2011). There are no reports on CBG tolerance to pinoxaden; however tolerance to highland (*Agrostis castellana* Boiss. and Reuter) and browntop (*Agrostis capillaris* L.) bentgrass has been observed (Syngenta Greencast 2010).

Registered formulations of pinoxaden (Axial® and Rescue®) contain the herbicide safener cloquintocet-mexyl in a 4:1 herbicide:safener ratio (Anonymous 2008, 2010b). A pro-herbicide, pinoxaden is metabolized to the herbicidally active compound (a 2-aryl-1,3-dione) by removal of a pivaloyl group via de-esterases in wheat and target weeds equally. Cloquintocet-mexyl accelerates the hydroxylation of this herbicidally active compound to inactive metabolites in winter wheat (*Triticum aestivum* L. 'Soisson') and barley (*Hordeum vulgare* L. 'conquest'), but not in perennial ryegrass and wild oats (*Avena fatua* L.) (Muehlebach et al. 2011). This resultant increase in selectivity renders cloquintocet-mexyl a valuable component of the herbicidal mixture.

Aryloxyphenoxypropionate ACCase-inhibiting herbicides have increased selectivity in cereals when applied with the safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl can increase selectivity of in cereal crops as well (Hatzios and Burgos 2004). These safeners are known to increase conjugation of herbicidally active fenoxaprop acid with glutathione in wheat and barley, but not weeds such as wild oats and crabgrass (Yaacoby et al. 1999). However, not all weeds are unaffected by safeners; fenchlorazole-ethyl and mefenpyr-

diethyl can reduce fenoxaprop-ethyl efficacy against blackgrass (*Alopecurus myosuroides* Huds.) (Cummins et al. 2009). In turfgrass, cloquintocet-mexyl can increase CBG tolerance to the *p*-hydroxyphenylpyruvate dioxygenase herbicide topramezone (Elmore et al. submitted). However, no other reports of increased turfgrass tolerance to herbicides from compounds used exclusively as herbicide safeners exist.

Considering that the few herbicide options for perennial ryegrass and roughstalk bluegrass control in CBG only exhibit marginal efficacy, investigating new herbicide options is warranted. The objective of this research was to evaluate CBG tolerance and weed control with pinoxaden alone and in combination with cloquintocet-mexyl, mefenpyr-diethyl and fenchlorazole-ethyl at different herbicide:safener ratios. Our hypothesis was that some safeners would increase CBG tolerance to pinoxaden, but they would also reduce efficacy against perennial ryegrass and roughstalk bluegrass. Additionally, we hypothesized that responses of CBG, perennial ryegrass and roughstalk bluegrass would vary at different herbicide:safener ratios.

MATERIALS AND METHODS

Plant Culture. Glasshouse experiments were conducted from 2012 to 2014 in Knoxville, TN (35° 57' N lat.) under ambient light. All plants were irrigated as needed to prevent wilt and grown in a peat moss, perlite and vermiculite growing medium (Fafard No. 2, Sun Gro Horticulture, Agawam, MA) and allowed to mature for at least 6 months prior to treatment.

Experiment 1: Identify Candidate Safeners. 'Penncross' CBG was seeded to 6.5-cm diameter cone-tainers (Stuewe and Sons, Tangent, OR) and maintained in a greenhouse at a 1.3 cm height using mechanical shears (Showmaster, Oster Professional Products, McMinnville, TN) for 6

months before treatment. During this time, CBG was fertilized on an as-needed basis using a complete (20N:20P₂O₅:20K₂O) fertilizer (Howard Johnson's Triple Twenty Plus Minors, Milwaukee, WI) at 25 kg N ha⁻¹ and irrigated as needed to prevent wilt. Beginning 10 days prior to treatment application, CBG was fertilized with a complete fertilizer at 10 kg N ha⁻¹ every 10 days for the duration of the experiment. Plants were hand-trimmed to a 1.3 cm height with scissors approximately 24 hours before treatment and mowing was then suspended for the duration of the experiment.

CBG was treated with safeners listed in Table 2.1 at a 5:1 safener:herbicide ratio 30 mins prior to application of pinoxaden (90 g ha⁻¹; 0.83 EC, Syngenta Professional Products, Greensboro, NC). Pinoxaden and the safener were applied to efficiently use a limited supply of pinoxaden. Comparisons were made to a non-treated control. Safeners in Table 2.1 were also applied to CBG alone at 750 g ha⁻¹ to assess phytotoxicity. All treatments were applied with non-ionic surfactant (Activator 90, Loveland Products Inc., Loveland, CO) at 0.25% v/v in 215 L ha⁻¹ of water carrier through a single flat-fan nozzle (8004 EVS; Spraying Systems Co., Roswell, GA) in a spray chamber (Generation III Research Track Sprayer, DeVries Manufacturing, Hollandale, MN). Naphthalic anhydride (NA) was dissolved in 3 ml of acetone prior to addition to appropriate spray solutions. Irrigation was withheld for 18 h after treatment application. Glasshouse air temperatures averaged 24 °C and ranged from 18 to 29 °C for the duration of each experimental run.

CBG injury was evaluated 1, 2 and 3 weeks after treatment (WAT) on a 0 (no injury) to 100% (complete leaf tissue necrosis) scale. Only CBG injury data collected 2 WAT will be presented, as treatment responses were most apparent at this time. CBG injury was also quantified via measurements of turfgrass cover via digital image analysis (DIA) using methods

modified from Richardson (2001) and Karcher and Richardson (2003). CBG cone-tainers were placed into a custom-constructed light box with a blue background and digitally photographed with a Canon G12 (Canon Inc., Japan) camera. Sigma Scan Pro Software (v. 5.0, SPSS. Inc., Chicago, IL) was used to determine the number of green pixels in each image. Green pixels were defined as those having a hue between 60 and 102 and saturation between 0 and 100 percent to exclude herbicide-injured leaves (defined as hue < 60; 103 to 134) and the blue background (hue range 135 to 153) from selection. Additionally, clipping yield was quantified 3 WAT to measure how pinoxaden affected the vigor of CBG. Henry and Hart (2004) also used this technique to assess CBG and velvet bentgrass injury from the ACCase-inhibitor fenoxaprop. Scissors were used to remove foliage above 1.3 cm. Harvested tissue was then placed in a drying oven at 80°C for 48 h and weighed. Clipping yields were transformed to a percentage of the non-treated control.

Treatments were arranged in a single factor treatment design with three replications. Herbicides were applied on 16 and 30 August 2012 for experimental runs A and B, respectively.

Experiment 2: Evaluate Safener Efficacy at Reduced Rates. Additional glasshouse research evaluated the efficacy of safeners identified as efficacious in Experiment 1. Cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were evaluated at 450, 225, 90, 68, 45, 23 or 0 g ha⁻¹ in combination with pinoxaden at 90 g ha⁻¹. CBG was treated with safeners 30 mins prior to pinoxaden application. Comparisons were made to a non-treated control. Treatments were arranged in a three (safener) by seven (safener rate), completely randomized factorial design with five replications. Plant culture, treatment application, and data collection were conducted in the same manner as Experiment 1. Treatments for experimental runs A and B were applied on 19

March and 11 October 2013. Glasshouse air temperatures averaged 23 °C and ranged from 16 to 32 °C during Run A. In Run B, air temperatures averaged 19 °C and reached a maximum of 28 °C, but a problem with the glasshouse heating system resulted in air temperatures as low as 7 °C for 4 to 6 h each night from 9 to 16 days after treatment.

Experiment 3: Comparing CBG, Roughstalk Bluegrass and Perennial Ryegrass Responses.

Based on results of Experiment 2, glasshouse research was conducted to evaluate the response of CBG, roughstalk bluegrass and perennial ryegrass to pinoxaden (90 g ha⁻¹) alone and in combination with the herbicide safeners cloquintocet-mexyl, mefenpyr-diethyl and fenchlorazole-ethyl at low or high rates (68 or 450 g ha⁻¹, respectively). Safeners were applied 30 mins prior to pinoxaden application. CBG, roughstalk bluegrass and perennial ryegrass were seeded to separate 10-cm diameter pots filled with growth media described previously. After grass emergence, pots were hand-thinned to contain five plants each. Plants were maintained at a 2.5 cm height for 6 months using mechanical shears. Plants were fertilized with the complete fertilizer described earlier at 49 kg N ha⁻¹ one week after germination, and also 4 and 2 weeks prior to treatment. Plants were also fertilized with complete fertilizer at 12 kg N ha⁻¹ 24 h before treatment and every 10 d after treatment. Plants were clipped to a 2.5-cm height with scissors 24 h before treatment and then mowing was suspended. Roughstalk bluegrass, perennial ryegrass and CBG averaged 9, 5, and 10 tillers per plant, respectively, at application. Treatments for Run A and B were applied on 7 and 14 May 2014, respectively.

Treatments were arranged in a three (safener) by two (safener rate), completely randomized factorial design with four replications. Non-treated and pinoxaden-treated (90 g ha⁻¹) controls were included for comparison. CBG injury, as well as perennial ryegrass and roughstalk

bluegrass control were evaluated 2 and 4 WAT as described previously. Clipping yields were collected 2 and 4 WAT as described previously and transformed to a percentage of the non-treated control. At 4 WAT, the number of surviving perennial ryegrass tillers were counted in each pot. Surviving tillers were defined as those that had green leaf shoots that had grown above the rest of the canopy.

Statistical Analysis. Model assumptions were tested through residual analysis (Shapiro–Wilk statistic) in SAS (Statistical Analysis Software, Inc., Cary, NC), and no transformations were needed. ANOVA, mean separations, and contrasts were conducted in SAS with main effects and all possible interactions tested using the appropriate expected mean square values as described by McIntosh (1983). Perennial ryegrass clipping yield data collected 4 WAT were subjected to an arcsine-square root transformation. However, non-transformed data are presented for clarity. Fisher’s protected LSD ($P \leq 0.05$) was used to separate means for experiments 1 and 2. For experiment 3, contrasts or paired t-tests were used to determine if responses observed on safener-treated plants differed ($\alpha \leq 0.05$) from pinoxaden-treated plants.

RESULTS AND DISCUSSION

Experiment 1: Identify Candidate Safeners. The effect of safener was significant for both CBG injury and clipping yield; but not DIA. However, DIA and visual injury were correlated ($r = -0.71$; $P < 0.0001$). Safeners applied alone did not cause CBG injury or clipping yield reduction and therefore were not included in the statistical analyses.

Pinoxaden alone caused 9% CBG injury and reduced clipping yield by 30% compared to the non-treated control (Table 2.2). The safeners cloquintocet-mexyl, mefenpyr-diethyl and

fenchlorazole-ethyl reduced pinoxaden injury to $\leq 3\%$. These safeners also ameliorated clipping yield reductions caused by pinoxaden. The safeners NA, isoxadifen and benoxacor had no effect on CBG injury or clipping yield reductions caused by pinoxaden. These data indicate cloquintocet-mexyl, mefenpyr-diethyl and fenchlorazole-ethyl can increase CBG tolerance to pinoxaden and warrant further investigation to determine optimal application rates.

Experiment 2: Evaluate Safener Efficacy at Reduced Rates. Safener rate-by-experimental run interactions were significant; therefore, runs will be presented separately. Safener rate-by-safener interactions were not significant in either experimental run; therefore data will be presented across safener rates and safeners where appropriate. DIA and visual injury were correlated in Run A ($r = -0.78$; $P < 0.0001$) and Run B ($r = -0.61$; $P < 0.0001$).

Herbicide Safener Efficacy (Combined across safener rates). In Run A, mefenpyr-diethyl reduced CBG injury from pinoxaden from 15 to 6%, more than cloquintocet-mexyl or fenchlorazole-ethyl (Table 3). Similarly, clipping yield of CBG treated with pinoxaden and mefenpyr-diethyl measured 62% of the non-treated, higher than pinoxaden applied with cloquintocet-mexyl (47%) or fenchlorazole-ethyl (51%). In Run B, pinoxaden applied alone only caused 8% injury and a 56% reduction in clipping yield; however, mefenpyr-diethyl was still the most effective safener as it reduced injury to 2% and clipping yields to only 78% of the non-treated. While mefenpyr-diethyl was the most effective safener, these responses indicate that all safeners can reduce CBG injury from pinoxaden.

Herbicide Safener Rates (Combined across safeners). The highest safener rate (450 g ha^{-1}) reduced CBG injury more than any other rate; this rate reduced CBG injury from 15% to 2% in Run A and 8 to 1% in Run B (Table 4). Compared to pinoxaden applied alone, all safener rates reduced CBG injury in Run A, but safener application at $\geq 68 \text{ g ha}^{-1}$ was required to both reduce CBG injury and increase clipping yield in Run B. Safener rates above 68 g ha^{-1} in Run B, did not affect CBG injury and clipping yield.

Other researchers have reported cloquintocet-mexyl and fenchlorazole-ethyl completely reduced wheat and barley injury from pinoxaden and fenoxaprop-ethyl at a 4:1 herbicide:safener ratio (Muehlebach et al. 2010; Yaacoby et al. 1991). While herbicide safeners reduced CBG injury from pinoxaden in our experiments, complete reduction of injury and clipping yield at high herbicide:safener ratios (4:1) similar to those commonly reported in cereal crops was not observed. However, other researchers have reported lower safener:herbicide ratios did not completely reduce wheat injury from ALS-inhibiting herbicides. Crooks et al. (2004) reported mefenpyr-diethyl at a lower 1:1 or 1:3 herbicide:safener ratio reduced wheat injury from 27 to 9% at 3 WAT with mesosulfuron-methyl and iodosulfuron-methyl, which supports the inclusion of mefenpyr-diethyl at a lower 1:2.15 herbicide:safener ratio in the cereal herbicide containing mesosulfuron-methyl and propoxycarbazone-sodium (Anonymous 2012b).

Experiment 3: Comparing CBG, Roughstalk Bluegrass and Perennial Ryegrass Responses.

CBG and perennial ryegrass response data were combined across experimental runs. Main effect interactions with experimental run were detected in roughstalk bluegrass data; therefore data from each experimental run will be presented separately. Main effects, but not their interactions were significant, Thus, only main effects will be presented for CBG and perennial ryegrass

responses as well as roughstalk bluegrass control. Safener-by-safener rate interactions were detected in roughstalk bluegrass control data and will be presented. Safener-by-safener rate interactions were not detected for CBG or roughstalk bluegrass clipping yield data.

CBG Responses. At 2 WAT, all safeners and safener rates increased CBG clipping yield and reduced injury compared to plants treated with pinoxaden only (Tables 2.5 and 2.6).

Cloquintocet-mexyl and mefenpyr-diethyl reduced CBG injury more than fenchlorazole-ethyl.

Pinoxaden alone reduced CBG clipping yield to 32% of the non-treated, but application in conjunction with either cloquintocet-mexyl or mefenpyr-diethyl increased yield to 69 and 75% of the non-treated. CBG injury from pinoxaden measured 25%, but was reduced to $\leq 5\%$ by cloquintocet-mexyl and mefenpyr-diethyl and 11% by fenchlorazole-ethyl. The high safener rate (450 g ha^{-1}) reduced injury and increased clipping yield more than the low (68 g ha^{-1}) rate. By 4 WAT, CBG clipping yields were similar for all safener treatments and not different from CBG treated with pinoxaden only. CBG injury was $< 5\%$ for all treatments at 4 WAT.

Perennial Ryegrass Responses. Neither safener treatment nor safener rate affected perennial ryegrass clipping yield or reduced control provided by pinoxaden at 2 WAT. Safener rates did not affect perennial ryegrass control at 4 WAT. Clipping yield, which was used to assess plant vigor, measured between 3 and 12% of the non-treated with perennial ryegrass control ranging from 83 to 95% for all treatments at 4 WAT. Perennial ryegrass treated with pinoxaden and mefenpyr-diethyl displayed greater perennial ryegrass control and a lower clipping yield than plants treated with pinoxaden and cloquintocet-mexyl at 4 WAT. Perennial ryegrass treated with pinoxaden and cloquintocet-mexyl also averaged seven surviving tillers per pot, compared to

only one in mefenpyr-diethyl-treated pots at 4 WAT (data not presented). Compared to perennial ryegrass treated with pinoxaden only, safeners did not affect perennial ryegrass control, clipping yield, or the number of surviving tillers at 4 WAT.

Roughstalk Bluegrass Responses. Mefenpyr-diethyl and cloquintocet-mexyl reduced roughstalk bluegrass control with pinoxaden and increased clipping yields at most evaluations (Tables 2.7 and 2.8). When treated with pinoxaden alone, roughstalk bluegrass control was 41 and 80% at 4 WAT in runs A and B, respectively; cloquintocet-mexyl and mefenpyr-diethyl decreased control to < 10% in Run A and < 65% in Run B. Clipping yield responses supported visual observations. The clipping yield of roughstalk bluegrass increased from 21% of the control with pinoxaden alone to 49 and 43% of the control with cloquintocet-mexyl and mefenpyr-diethyl respectively, at 2 WAT in Run A. By 4 WAT in Run A, clipping yields in roughstalk bluegrass treated with mefenpyr-diethyl and cloquintocet-mexyl were 107 and 113% of the control. Pinoxaden was more efficacious at 4 WAT in Run B than in Run A, but clipping yields of roughstalk bluegrass treated with mefenpyr-diethyl and cloquintocet-mexyl were greater than those in roughstalk bluegrass treated with pinoxaden alone. Generally, fenchlorazole-ethyl did not reduce pinoxaden efficacy or clipping yield. High (450 g ha^{-1}) safener rates decreased control more than low rates at 2 WAT in both experimental runs and 4 WAT in Run A. Safeners can antagonize weed control in cereal crops as well. Mefenpyr-diethyl and fenchlorazole-ethyl can decrease fenoxaprop-ethyl efficacy against blackgrass, a problematic weed of cereal crops in Europe (Cummins et al. 1999). However, the combination of fenoxaprop-p-ethyl and mefenpyr-diethyl is registered for blackgrass control in cereals (Anonymous 2011).

Conclusions. These data indicate that safeners traditionally used in cereal crops (cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl) to reduce injury from ACCase-inhibiting herbicides reduced CBG injury from pinoxaden more than safeners used in corn (benoxacor, isoxadifen-ethyl, and naphthalic anhydride). Reductions in CBG injury increased with safener rate and depend on the safener selected. Cloquintocet-mexyl and mefenpyr-diethyl more effectively reduced CBG injury from pinoxaden than fenchlorazole-ethyl. These safeners did not affect pinoxaden efficacy against perennial ryegrass, but reduced efficacy against roughstalk bluegrass. Since reductions in roughstalk bluegrass control, especially at low (68 g ha^{-1}) safener rates were offset by similar reductions in CBG injury, these data suggest safeners will not reduce pinoxaden selectivity for roughstalk bluegrass in CBG. However, more research is needed to optimize pinoxaden:safener ratios and rates.

Future research should evaluate pinoxaden in combination with cloquintocet-mexyl or mefenpyr-diethyl in field experiments. Since CBG injury and roughstalk bluegrass control are dependent on safener rate, multiple safener and herbicide rates should be evaluated in different climates and seasons. The effect of different adjuvants on efficacy and selectivity of pinoxaden-safener combinations should also be evaluated as our experiments only evaluated NIS. The methylated rapeseed oil-based adjuvant Adigor® improves pinoxaden absorption and efficacy more than non-ionic surfactants, likely a result of its effects on leaf cuticle plasticity (Chitband et al. 2013; Mohassel et al. 2011; Muehlebach et al. 2011; Penner 2000).

These experiments only evaluated single herbicide applications in a glasshouse. While they can be used to assess relative efficacy of different treatments, field research will be needed to understand whether sequential applications are required to control roughstalk bluegrass and perennial ryegrass in CBG. Even if multiple applications are required for complete perennial

ryegrass and roughstalk bluegrass control, pinoxaden-safener combinations may be a viable option for turfgrass managers if more research is conducted.

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APPENDIX
TABLES AND FIGURES

Table 2.1. Safeners applied in Experiment 1 to ‘Penncross’ creeping bentgrass in a glasshouse in Knoxville, TN in 2013. Safeners were applied at 450 g ha⁻¹, 30 mins prior to pinoxaden treatment at 90 g ha⁻¹.

Common name	Trade name	Formulation	Manufacturer
Naphthalic anhydride ^a	N/A	> 97% Tech.	Acros Organics; Pittsburgh, PA
Isoxadifen-ethyl	N/A	50 WDG	DuPont Ag Products; Wilmington, DE
Benoxacor	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC
Cloquintocet-mexyl	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC
Fenchlorazole-ethyl	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC
Mefenpyr-diethyl	N/A	1.6 EC	Arysta Lifescience North America LLC; Cary, NC

^aSafeners were applied with non-ionic surfactant at 0.25% v/v.

Table 2.2. ‘Penncross’ creeping bentgrass injury and clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2013. Pinoxaden was applied at 90 g ha⁻¹. Safeners were applied at 450 g ha⁻¹ 30 mins prior to pinoxaden. Creeping bentgrass visual injury was evaluated 2 WAT on a 0 (no injury) to 100% (complete control) scale. Clippings were collected 3 WAT, dried, weighed and transformed to a percentage of the non-treated control.

Safener	Injury	Clipping yield
	(2 WAT ^b)	(3 WAT)
	—%—	—% of non-treated—
Naphthalic anhydride ^a	8	95
Isoxadifen-ethyl	7	90
Benoxacor	8	75
Cloquintocet-mexyl	2	106
Fenchlorazole-ethyl	2	120
Mefenpyr-diethyl	3	118
None	9	70
LSD _{0.05}	5	27

^aAll treatments were applied with non-ionic surfactant at 0.25% v/v.

^bAbbreviation: WAT, weeks after treatment.

Table 2.3. ‘Pennncross’ creeping bentgrass injury and clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2013. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were applied 30 mins prior to pinoxaden (90 g ha⁻¹) application at 450, 225, 90, 68, 45 or 23 g ha⁻¹. Creeping bentgrass visual injury was evaluated at 2 WAT on a 0 (no injury) to 100% (complete control) scale. Clippings were collected 3 WAT, dried, weighed and transformed to a percentage of the non-treated control. Data are combined across safener rates.

Safener	Injury		Clipping yield	
	Run A	Run B	Run A	Run B
	—%—		—% of non-treated—	
cloquintocet-mexyl ^a	9	4	47	61
fenchlorazole-ethyl	8	5	51	67
mefenpyr-diethyl	6	2	62	78
None	15	8	26	56
LSD _{0.05}	2	2	6	11

^aAll treatments were applied with non-ionic surfactant at 0.25% v/v.

Table 2.4. ‘Penncross’ creeping bentgrass injury and clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2013. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were applied at 450, 225, 90, 68, 45, 23 or 0 g ha⁻¹ 30 mins prior to pinoxaden (90 g ha⁻¹) application. Creeping bentgrass visual injury was evaluated at 2 WAT on a 0 (no injury) to 100% (complete control) scale. Clippings were collected 3 WAT, dried, weighed and transformed to a percentage of the non-treated control. Data are combined across herbicide safeners.

Safener Rate	Injury		Clipping yield	
	Run A	Run B	Run A	Run B
—g ha ⁻¹ —	——%——		—% of non-treated—	
450 ^a	2	1	70	77
225	6	4	57	68
90	7	4	59	66
68	10	2	51	65
45	9	5	46	70
23	11	7	40	66
0	15	8	26	56
LSD _{0.05}	3	3	9	17

^aAll treatments were applied with non-ionic surfactant at 0.25% v/v.

Table 2.5. ‘Penncross’ creeping bentgrass and perennial ryegrass clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were applied at High or Low rates (225 or 68 g ha⁻¹, respectively) 30 mins prior to pinoxaden (90 g ha⁻¹) application. Clippings were collected 2 and 4 weeks after treatment, dried, weighed and transformed to a percentage of the non-treated control. Data are combined across two experimental runs.

Safener	Creeping Bentgrass						Perennial Ryegrass					
	2 WAT ^a			4 WAT			2 WAT			4 WAT		
	% of control		Contrast	% of control		Contrast	% of control		Contrast	% of control		Contrast
Cloquintocet-mexyl ^b	69	a ^c	*** ^d	123	a	NS	27	a	NS	12	a	NS
Fenchlorazole-ethyl	56	b	**	138	a	NS	30	a	NS	5	ab	NS
Mefenpyr-diethyl	75	a	***	129	a	NS	30	a	NS	3	b	NS
None	32	-	-	133	-	-	31	-	-	6	-	-
Safener Rate												
High	76	a	***	126	a	NS	26	a	NS	6	a	NS
Low	57	b	**	134	a	NS	32	a	NS	8	a	NS

^a Abbreviations: WAT, weeks after treatment.

^b All treatments were applied with non-ionic surfactant at 0.25% v/v.

^c Means followed by the same letter are not statistically different ($P \leq 0.05$)

^d Contrasts were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. NS, non-significant; **, ***, significant when $\alpha \leq 0.01$ and 0.001, respectively.

Table 2.6. Creeping bentgrass injury and perennial ryegrass control after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were applied at high or low rates (225 or 68 g ha⁻¹, respectively) 30 mins prior to pinoxaden (90 g ha⁻¹) application. Control was evaluated visually on a 0 (no injury or control) to 100% (complete control) scale 2 and 4 weeks after treatment. Data were combined across experimental runs.

Safener	Creeping Bentgrass						Perennial Ryegrass					
	2 WAT ^a			4 WAT			2 WAT			4 WAT		
	—% injury—		Contrast	—% injury—		Contrast	—% Control—		Contrast	—% Control—		Contrast
Cloquintocet-mexyl ^b	5	b ^c	*** ^d	2	a	NS	60	a	NS	83	b	NS
Fenchlorazole-ethyl	11	a	***	2	a	NS	59	a	NS	92	a	NS
Mefenpyr-diethyl	3	b	***	0	a	**	64	a	*	95	a	NS
None	25	-	-	4	-	-	55	-	-	89	-	
Safener Rate												
High	4	b	***	1	a	*	61	a	NS	89	a	NS
Low	8	a	***	2	a	NS	61	a	NS	90	a	NS

^aAbbreviations: WAT, weeks after treatment.

^bAll treatments were applied with non-ionic surfactant at 0.25% v/v.

^cMeans followed by the same letter are not statistically different ($P \leq 0.05$)

^dContrasts were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. NS, non-significant; *, **, ***, significant when $\alpha \leq 0.05$, 0.01 and 0.001, respectively.

Table 2.7. Roughstalk bluegrass control after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were applied at high or low rates (225 or 68 g ha⁻¹, respectively) 30 mins prior to pinoxaden (90 g ha⁻¹) application. Control was evaluated visually on a 0 (no control) to 100% (complete control) scale 2 weeks after treatment. Data from two experimental runs are presented separately.

Safener	Rate	2 Weeks After Treatment						4 Weeks After Treatment					
		Run A			Run B			Run A			Run B		
		—% Control—		T-test	—% Control—		T-test	—% Control—		T-test	—% Control—		T-test
Cloquintocet-mexyl ^a	High	14	b ^b	** ^c	33	b	**	5	ab	**	15	b	**
	Low	25	ab	**	69	a	NS	4	ab	**	62	a	NS
Fenchlorazole-ethyl	High	34	a	NS	62	a	NS	25	a	NS	52	a	*
	Low	28	ab	**	68	a	NS	18	ab	NS	76	a	NS
Mefenpyr-diethyl	High	14	b	**	25	b	***	0	b	***	11	b	***
	Low	32	a	*	59	a	NS	4	ab	**	50	a	NS
None	-	49	-	-	68	-	-	41	-	-	80	-	-

^aAll treatments were applied with non-ionic surfactant at 0.25% v/v.

^bMeans followed by the same letter are not statistically different ($P \leq 0.05$)

^c T-tests were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. NS, non-significant; *, **, ***, significant when $\alpha \leq 0.05$, 0.01 and 0.001, respectively.

Table 2.8. Roughstalk bluegrass clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were applied at high or low rates (225 or 68 g ha⁻¹, respectively) 30 mins prior to pinoxaden (90 g ha⁻¹) application. Clippings were collected 2 and 4 weeks after treatment, dried, weighed and transformed to a percentage of the non-treated control. Data from two experimental runs are presented separately.

Safener	2 Weeks After Treatment						4 Weeks After Treatment					
	Run A			Run B			Run A			Run B		
	—% of control—		T-test	—% of control—		T-test	—% of control—		T-test	—% of control—		T-test
Cloquintocet-mexyl ^a	49	a ^b	** ^c	32	a	*	107	a	**	80	a	**
Fenchlorazole-ethyl	31	b	NS	22	a	NS	88	a	NS	42	b	NS
Mefenpyr-diethyl	43	ab	*	27	a	NS	113	a	**	70	a	***
None	21	-	-	19	-	-	69	-	-	24	-	-
Safener Rate												
High	46	a	**	32	a	*	100	a	**	85	a	***
Low	36	a	NS	21	b	NS	104	a	**	43	b	NS

^aAll treatments were applied with non-ionic surfactant at 0.25% v/v.

^bMeans followed by the same letter are not statistically different ($P \leq 0.05$)

^cContrasts were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. NS, non-significant; *, **, ***, significant when $\alpha \leq 0.05$, 0.01 and 0.001, respectively.

**CHAPTER 3: CYTOCHROME P450 INHIBITORS AFFECT CREEPING
BENTGRASS TOLERANCE TO TOPRAMEZONE**

This chapter is based on a paper that will be submitted for publication by Matthew Elmore, James Brosnan, Gregory Armel, Dean Kopsell, Michael Best, Thomas Mueller and John Sorochan:

Elmore, M.T., J.T. Brosnan, G.R. Armel, D.A. Kopsell, M.D. Best, T.C. Mueller and J.C. Sorochan. 2014. Cytochrome P450 Inhibitors Reduce Creeping Bentgrass (*Agrostis stolonifera*) Tolerance to Topramezone. Pesticide Biochemistry and Physiology.

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

ABSTRACT

Creeping bentgrass (*Agrostis stolonifera* L.) is moderately tolerant to the *p*-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide topramezone. However, the contribution of topramezone metabolism to this tolerance is unknown. Experiments were conducted to determine if known cytochrome P450 monooxygenase inhibitors 1-aminobenzotriazole (ABT) and malathion influence creeping bentgrass tolerance to topramezone. Creeping bentgrass in hydroponic culture was treated with ABT (70 μM) or malathion (70 μM and 1000 g ha⁻¹) prior to topramezone (8 g ha⁻¹) application to foliage. Topramezone injury to creeping bentgrass increased from 22% when applied alone to 79 and 41% when applied with malathion or ABT, respectively. Cloquintocet (70 μM and 1000 g ha⁻¹) reduced topramezone injury to 1%. Cloquintocet mitigated the synergistic effects of ABT more

than those of malathion. Visible responses were supported by chlorophyll fluorescence yield and creeping bentgrass biomass responses. Responses to ABT and malathion suggest that creeping bentgrass tolerance to topramezone is influenced by cytochrome P450-catalyzed metabolism. Future research should determine the contributions of cytochrome P450 monooxygenases and glutathione-*s*-transferases to topramezone metabolism in creeping bentgrass.

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INTRODUCTION

Creeping bentgrass (*Agrostis stolonifera* L.) is the most widely used cool-season turfgrass on golf course fairways and tees in the United States [1]. Crabgrass (*Digitaria* spp.), goosegrass (*Eleusine indica* L. Gaertn.) and bermudagrass (*Cynodon* spp.) are difficult to control weeds that disrupt the functional and aesthetic quality of creeping bentgrass turf [2]. Herbicide options for weed control in creeping bentgrass are limited. Herbicides such as fenoxaprop-*p*-ethyl and quinclorac often cannot be applied at rates that provide acceptable weed control as they are too injurious to creeping bentgrass [3-8].

Topramezone is a pyrazolone *p*-hydroxyphenylpyruvate dioxygenase (HPPD; EC 1.13.11.27)-inhibiting herbicide registered for use in corn (*Zea mays* L.) and turfgrass [9,10]. When applied between 12 and 37 g ha⁻¹, topramezone can control multi-tiller crabgrass and goosegrass, while sequential applications can suppress bermudagrass [11-13]. Topramezone is registered for application to most C₃ turfgrass species at ≤ 37 g ha⁻¹, but application to creeping bentgrass can cause injury at 6 to 37 g ha⁻¹ [10,11,13,14]. However, if creeping bentgrass injury from topramezone could be reduced without sacrificing weed control, it would be an excellent tool for weed management.

Our previous research demonstrated that creeping bentgrass is more tolerant to topramezone than large crabgrass or goosegrass; the herbicide safener cloquintocet-mexyl further increases this tolerance and does not reduce topramezone efficacy against large crabgrass and goosegrass [15]. Desirable herbicide safeners protect graminaceous crop plants from herbicide injury more than target weeds, increasing the margin of selectivity [16]. Safeners function predominately by increasing activity of cytochrome P450 monooxygenases and transferases that catalyze Phase I or II reactions involved in herbicide metabolism [16-19]. Cloquintocet-mexyl is

used commercially in cereal crops to increase selectivity of acetyl-CoA carboxylase (EC 6.4.1.2) and acetolactate synthase (EC 2.2.1.6) inhibiting herbicides.

Rapid N-demethylation of topramezone to an inactive metabolite has been attributed to excellent corn tolerance (Figure 3.1)[9]. However, other research suggests there may be an alternative route of topramezone metabolism. A mutation of an allele coding for P450 enzymes in corn hybrids reduces tolerance to the HPPD-inhibitors mesotrione and tembotrione, especially if the hybrid is homozygous for the non-functional allele. Interestingly, while homozygous hybrids were injured >50% by mesotrione and tembotrione, topramezone caused no injury to any of the hybrids tested [20]. Mesotrione and presumably tembotrione undergo a P450-mediated hydroxylation at the 4-position on the cyclohexandione ring [21]. This suggests that a different metabolic mechanism may confer corn tolerance to topramezone. Grossman and Ehrhardt [9] demonstrated that differential affinity of topramezone for corn and grassy weed HPPD was not the main mechanism conferring corn tolerance. The mechanism of creeping bentgrass tolerance to topramezone is unknown. Additionally, the mechanism by which cloquintocet-mexyl increases creeping bentgrass tolerance to topramezone is unknown. Cloquintocet-mexyl can increase glutathione-*s*-transferase (GST) activity in wheat (*Triticum aestivum* L.) and has also been reported to increase pyridinyl N-glucosylation of clodinafop-propargyl [22,23]. Topramezone N-glycosylation via N-glucosyltransferase at the un-saturated pyrimidinyl N may occur independent of P450-catalyzed metabolism. Metabolism may also proceed via O-glucosylation at hydroxyl groups; cloquintocet can increase O-glycosyltransferase activity in wheat [24].

Cytochrome P450 inhibitors such as 1-aminobenzotriazole (ABT), piperonyl butoxide (PBO), and malathion are commonly used to determine if P450-catalyzed reactions affect plant tolerance to herbicides [18,25]. Herbicides such as chlorotoluron and metflurazon can be N-

demethylated to their primary metabolites. These N-demethylations are thought to be P450-catalyzed [26]. ABT can reduce metabolism and increase the toxicity of chlorotoluron to wheat and resistant biotypes of *Lolium rigidum*, *Bromus tectorum* and *Alopecurus myosuroides* [27-29]. However, ABT synergizes chlorotoluron by inhibiting aryl ring alkyl hydroxylation, and does not affect N-demethylation [28,30]. These research reports suggest that ABT does not inhibit N-demethylase activity. However, ABT can inhibit the N-demethylation of metflurazon to the herbicidally active norfluazon in unicellular green algae (*Chlorella fusca*) [31]. Malathion can synergize ALS-inhibiting and acetyl-CoA carboxylase-inhibiting herbicides that can be metabolized via hydroxylation [32-36]. However, there are no reports on plant tolerance to topramezone in the presence of P450 inhibitors.

The objective of this experiment was to determine if creeping bentgrass tolerance to topramezone is affected by P450 inhibitors in the presence and absence of cloquintocet-mexyl. Our hypothesis was that the role of P450 topramezone metabolism in creeping bentgrass is minor compared to other routes. Thus, creeping bentgrass tolerance to topramezone would be reduced only slightly by the P450 inhibitors ABT and malathion and the effects of these P450 inhibitors would be offset by cloquintocet-mexyl, which can increase plant metabolism of herbicides through non-P450-catalyzed routes.

MATERIALS AND METHODS

Plant material and growing conditions

Plants were grown and experiments were conducted in a glasshouse at the University of Tennessee (Knoxville, TN; 35° 53' N lat.). Creeping bentgrass (*Agrostis stolonifera* L. c.v. 'Pennncross') seeds were planted into cone-tainers (3.8 cm diameter 20 cm depth; Stuewe and

Sons, OR, USA) filled with a peat moss, perlite and vermiculite growing medium (Fafard No. 2, Sun Gro Horticulture, MA, USA). After germination, cone-tainers were hand-thinned to contain one plant each. Plants were fertilized monthly using a complete (20N:20P₂O₅:20K₂O) (Howard Johnson's Triple Twenty Plus Minors, WI, USA) fertilizer at 25 kg N ha⁻¹, irrigated as needed to prevent wilt and maintained at a 2.5 cm height of cut with scissors twice weekly.

After six months of growth, roots were washed to completely remove the peat-based growth media and cut to a uniform 15 cm length to stimulate new root growth from the plant crown. The roots were then inserted through 7 mm holes in the lid of a 23 cm diameter polyethylene tub filled with 7 L of half-strength Hoagland nutrient solution [37]. Deionized water was added as needed to maintain a 7 L volume. The exterior of the tubs and lids were covered in silver paint to prevent algal growth in the nutrient solution. A blower fan was used to pump ambient air through Tygon® (Saint-Gobain Performance Plastics, OH, USA) tubing and a 2 cm diameter spherical airstone (Rolf C. Hagen Corp., MA, USA) submerged in the nutrient solution. Each polyethylene tub comprised an experimental unit and contained three creeping bentgrass plants.

Herbicide, P450-inhibitor, and Safener Treatment

After creeping bentgrass remained in hydroponic culture for 10 days, the cytochrome P450 inhibitors ABT (Alfa Aesar, MA, USA) and malathion (50% EC, Spectrum Brands, WI, USA), as well as the herbicide safener cloquintocet-mexyl (Bosche Scientific, NJ, USA) were added to the nutrient solution in appropriate amounts to achieve a 70 µM concentration. Optimum absorption and translocation of ABT is thought to occur through plant roots [39]. Conversely, cloquintocet-mexyl and malathion penetrate leaf cuticles well and are usually

applied to leaf tissue to determine their effects on herbicides [18, 38]. However, preliminary research determined that cloquintocet-mexyl added to nutrient solution reduced injury from topramezone more effectively than foliar applications. Therefore, in addition to their inclusion in nutrient solution, cloquintocet-mexyl and malathion were also applied to creeping bentgrass foliage at 28 and 1000 g ha⁻¹, respectively, 22 hours after their addition to the nutrient solution and two hours prior to topramezone application. The cloquintocet-mexyl rate was based on our previous research and the malathion rate is commonly used in investigations to determine mechanisms of herbicide resistance [15,32].

The inhibitors and safener compounds were arranged into six treatments as follows: (1) ABT; (2) malathion; (3) cloquintocet-mexyl; (4) ABT + cloquintocet-mexyl; (5) malathion + cloquintocet-mexyl; and (6) Non-treated control. These treatments were applied alone or in conjunction with topramezone (BAS 670H 2.8 SC; BASF Corp., NC, USA) at 8 g ha⁻¹ to creeping bentgrass foliage. Preliminary research suggested this topramezone dose would be sub-lethal. Treatments were arranged in a randomized complete block design with three replications and the experiment was repeated in time.

ABT, malathion and cloquintocet-mexyl were dissolved in 7 mL dimethyl sulfoxide and added to 7 L of nutrient solution 24 hours before topramezone application. The ABT and malathion concentrations were based on the methods of previous research evaluating the effects of P450 inhibitors on chlorotoluron metabolism [38,39]. Dimethyl sulfoxide was also added to non-treated nutrient solutions.

Foliar applications of topramezone, malathion and cloquintocet-mexyl were all performed separately, but in the same manner. All treatments were applied with non-ionic surfactant (Activator 90. Loveland Products Inc., Loveland, CO) at 0.25% v/v in 215 L ha⁻¹ of

water carrier through a single flat-fan nozzle (8004 EVS; Spraying Systems Co., Roswell, GA) in a spray chamber (Generation III Research Track Sprayer. DeVries Manufacturing, MN, USA). To prevent runoff of the spray solution from the polyethylene lid into the nutrient solution, a temporary paper backing lined with plastic was fitted between the creeping bentgrass foliage and lid and removed after spray applications.

Evaluating Treatment Effects

Before visible symptoms of topramezone injury were observed, chlorophyll fluorescence yield (F_v/F_m) was assessed 2 days after treatment (DAT). Other researchers [40-42] have used F_v/F_m for a quantitative measurement of the effects of HPPD-inhibiting herbicides, as it is correlated to reductions in chlorophyll and carotenoid concentrations; these reductions are an indirect, but consequential mechanism of HPPD-inhibition. F_v/F_m was measured using a pulse-modulated fluorometer (OS1-FL, Opti-sciences, Inc., Hudson, NH) by subtracting F_o , the minimal level of fluorescence, from F_m , the maximum level of fluorescence and dividing this difference by F_m [42]. Four measurements on each of the three plants in each experimental unit comprised of 12 subsamples that were averaged to determine a mean value.

Visible bleaching and/or necrosis of creeping bentgrass leaf tissue were evaluated at 7 and 10 DAT on a 0 (no bleaching) to 100% (complete leaf bleaching or necrosis) scale. Only data collected 10 DAT will be presented, as treatment responses were most apparent at this time.

Biomass was also collected 10 DAT. Scissors were used to remove all stem, leaf, and stolon tissue. The harvested tissue from all three plants in each experiment unit was combined and placed in a drying oven at 80°C for 48 h and weighed. Biomass data were transformed to a percentage of the non-treated control to determine treatment effects on overall plant vigor.

Statistical Analysis

Treatments were arranged in a single-factor randomized complete block design with three replications. Plants that were treated with P450 inhibitors or cloquintocet but not topramezone were analyzed separately and compared to the non-treated control. The non-treated control was also included in the analysis for chlorophyll fluorescence yield and biomass of plants treated with topramezone but removed for analysis of visible injury. Model assumptions were tested through residual analysis (Shapiro–Wilk statistic) in SAS (Statistical Analysis Software, Inc., Cary, NC), and no transformations were needed. ANOVA, mean separations, and contrasts were conducted in SAS with main effects and all possible interactions tested using the appropriate expected mean square values as described by McIntosh [43]. Fisher's protected LSD ($P \leq 0.05$) was used to separate means.

RESULTS AND DISCUSSION

Treatment interactions with experimental run were not detected ($P \leq 0.05$); therefore, data were combined across runs. For plants treated with topramezone, safener and P450 inhibitor treatments had a significant effect of chlorophyll fluorescence yield, biomass and visible injury ($P \leq 0.01$).

Chlorophyll fluorescence yield (F_v/F_m) responses

Malathion, ABT, cloquintocet-mexyl or combinations thereof did not affect F_v/F_m in the absence of topramezone (data not presented). Regardless of P450 inhibitor or safener inclusion, topramezone reduced the F_v/F_m compared to the non-treated control (Figure 3.2). When applied alone or to plants treated with ABT or malathion, topramezone reduced the F_v/F_m similarly by >

25%. Among plants treated with topramezone, those also treated with cloquintocet-mexyl had a higher F_v/F_m than others. Plants treated with cloquintocet-mexyl alone had a higher F_v/F_m than those treated with the combination of cloquintocet-mexyl and malathion. Visible injury symptoms were not apparent at 2 days after treatment when F_v/F_m was measured.

Visible responses 10 days after treatment

Topramezone applied alone caused 22 % visible injury, mostly in the form of bleaching, which is characteristic of HPPD-inhibiting herbicide application (Figure 3.3). Malathion increased injury from topramezone to 79%, more than that observed from any other treatment. Both visible bleaching and bleaching-induced necrosis were observed on plants treated with malathion. ABT also increased topramezone injury, but injury was less than that observed with malathion. Plants treated with cloquintocet-mexyl alone displayed only 1% injury, which was similar to that of the ABT and cloquintocet-mexyl combination. Topramezone caused less injury in plants treated with cloquintocet-mexyl + malathion than with malathion alone. Malathion, ABT, cloquintocet-mexyl or combinations thereof did not cause any visible injury in the absence of topramezone (data not presented).

Biomass responses 10 days after treatment

Leaf biomass of plants treated with topramezone alone measured 58% of the non-treated control. Compared to topramezone alone, biomass was lower in plants treated with malathion and topramezone. Biomass of plants treated with cloquintocet-mexyl or cloquintocet-mexyl + ABT were in the highest statistical category. When applied alone, malathion, ABT, cloquintocet-

mexyl or combinations thereof did cause any leaf biomass reductions compared to the non-treated control (data not presented). Leaf biomass data support visible injury responses.

The reductions in chlorophyll fluorescence yield and leaf tissue biomass concomitant with increases in visible bleaching and necrosis caused by both malathion and ABT suggest P450 monooxygenases influence topramezone metabolism in creeping bentgrass. Visible responses demonstrate malathion is a more potent synergist of topramezone in creeping bentgrass than ABT. Cloquintocet-mexyl completely eliminated creeping bentgrass injury from topramezone, supporting responses observed in our previous research [12]. Cloquintocet-mexyl antagonized the effects of malathion or ABT on topramezone, but the implications of this are not clear. It is possible cloquintocet-mexyl increased topramezone metabolism through mechanisms not catalyzed by P450 monooxygenases; cloquintocet-mexyl is known to increase pyridinyl N-glucosylation of clodinafop in wheat [22]. It is also possible that P450 monooxygenase activity was higher in plants treated with cloquintocet-mexyl even in the presence of ABT or malathion, or that P450's induced were not inhibited by malathion and ABT. Yun et al. [44] evaluated the effects of the P450 inhibitor PBO alone and in combination with safeners on the activity of microsomal pyrazosulfuron-ethyl *O*-demethylase, which metabolizes pyrazosulfuron-ethyl in rice (*Oryza sativa* L.). They found that PBO inhibited 63% of pyrazosulfuron-ethyl *O*-demethylase activity when in combination with the herbicide, but only 19% inhibition was achieved when PBO was in combination with the herbicide and the safener. The researchers suggested that this was because the herbicide and safeners induce different P450 enzymes.

Future research should evaluate topramezone metabolite formation in the presence of cloquintocet-mexyl and malathion. Additionally, microsomal assays could be used to qualify

P450's with activity on topramezone in tolerant grasses such as Kentucky bluegrass (*Poa pratensis* L.) compared to those of creeping bentgrass and susceptible weeds such as goosegrass.

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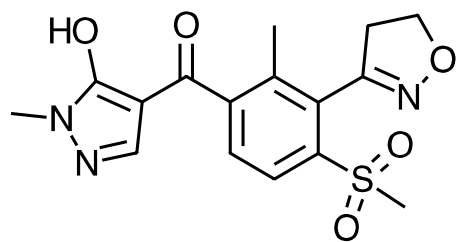
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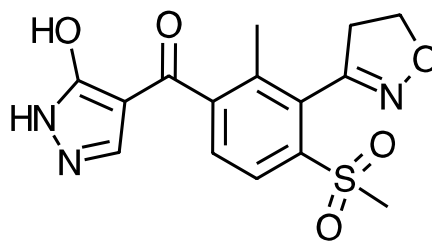
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APPENDIX
TABLES AND FIGURES



Topramezone



Topramezone-desmethyl

Figure 3.1. Topramezone and its primary metabolite, topramezone-desmethyl as proposed by Grossman and Ehrhardt (2007).

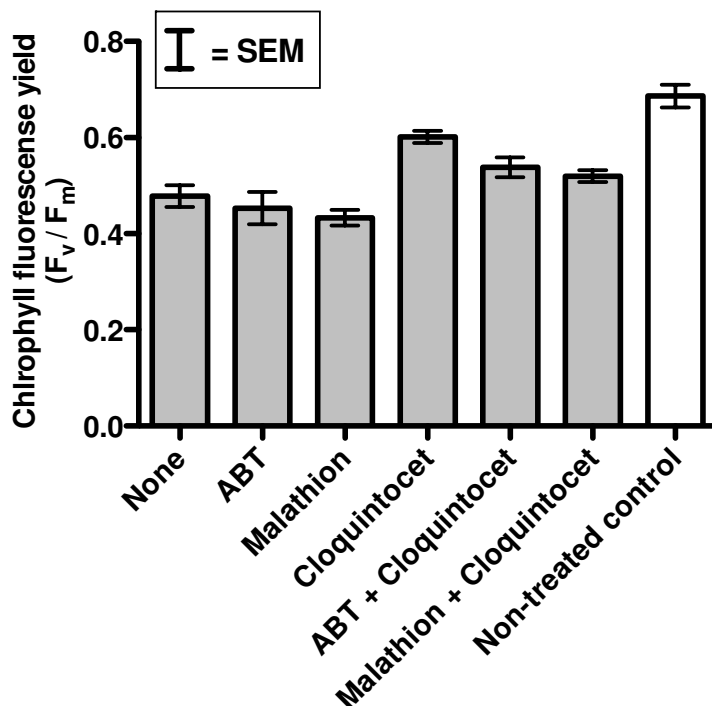


Figure 3.2. Chlorophyll fluorescence yield (F_v/F_m) of creeping bentgrass (*Agrostis stolonifera* L.) leaf tissue two days after treatment with topramezone (8 g ha^{-1}) alone or in combination with the cytochrome P450 monooxygenase inhibitors 1-aminobenzotriazole (ABT) or malathion. Creeping bentgrass was also treated with the herbicide safener cloquintocet-mexyl (cloquintocet) alone and in combination with ABT or malathion. A non-herbicide treated control is included for comparison. Standard error of the mean is used to separate means.

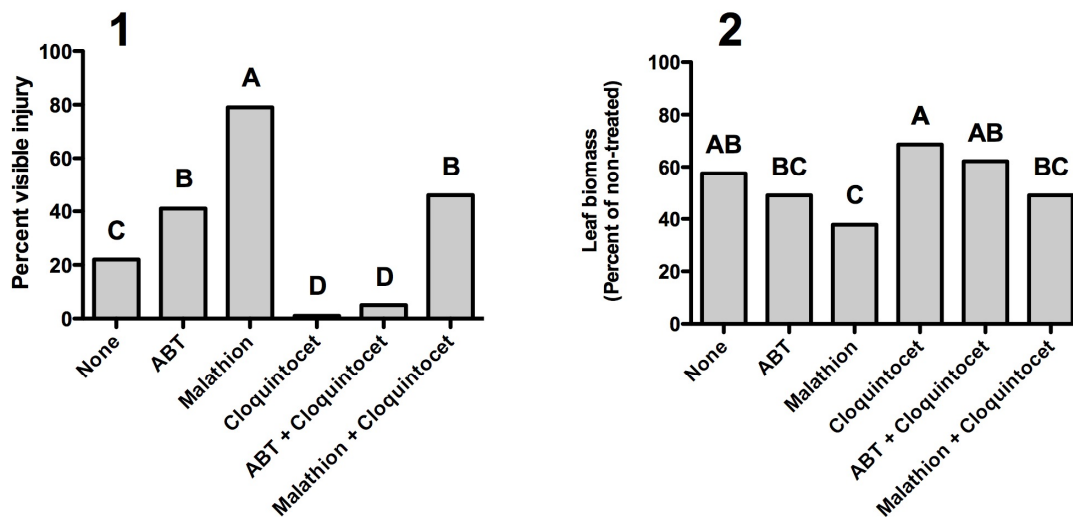


Figure 3.3. Visible injury (1) and biomass (2) of creeping bentgrass (*Agrostis stolonifera* L.) leaf tissue 10 days after treatment with topramezone (8 g ha^{-1}) in combination with the cytochrome P450 monooxygenase inhibitors 1-aminobenzotriazole (ABT) or malathion. Creeping bentgrass was also treated with the herbicide safener cloquintocet-mexyl (cloquintocet) alone or in combination with ABT or malathion. Creeping bentgrass visible injury was evaluated on a 0 (no injury) to 100% (complete bleaching or necrosis) scale. Biomass data were transformed to a percentage of the non-treated control. Columns containing the same letter are not statistically different ($\alpha \leq 0.05$).

**CHAPTER 4: SYNTHESIS AND EFFICACY OF TRIKETONE
DERIVATIVES AGAINST GRAMINACEOUS WEEDS**

This chapter is based on a paper that will be submitted for publication by Matthew Elmore, James Brosnan, Gregory Armel, Michael Best, Joseph Thomas, and Jose Vargas

Elmore, M.T., G.R. Armel, J.T. Brosnan, M.D. Best, J.W. Thomas and J.J. Vargas. 2014.
Herbicidal Efficacy of Triketone Derivatives Against Graminaceous Weeds.
Journal of Pesticide Science.

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

ABSTRACT

Triketone *p*-hydroxyphenylpyruvate dioxygenase inhibitors were synthesized and evaluated for their herbicidal efficacy. Previous research suggests that 2-benzoyl-1,3-cyclohexanedione compounds with alkyl substitutions on the cyclohexane moiety have enhanced efficacy against grassy weeds. However, there is no information describing how the efficacy of these alkyl-substituted compounds varies among grass species. We synthesized 2-(2,4-dichlorobenzoyl)cyclohexane-1,3-dione compounds with various alkyl substituents on the cyclohexandione moiety to understand their efficacy against grassy weeds of turfgrass systems such as *Digitaria sanguinalis*, *Eleusine indica*, and *Poa annua*. The 5,5-dimethyl-substituted compound had efficacy against *Eleusine indica* and did not cause injury to desirable turfgrass species *Poa pratensis* and *Agrostis stolonifera*.

INTRODUCTION

The 2-benzoylcyclohexane-1,3-diones, commonly referred to as triketones, represent a chemical class of herbicides that inhibit the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD) in susceptible plants¹⁻²⁾ (Figure 1). HPPD inhibition prevents conversion of *p*-hydroxyphenylpyruvate (HPP) to homogentisate, a precursor to plastoquinone, an essential cofactor for phytoene desaturase³⁾. A smaller plastoquinone pool results in reduced phytoene desaturase activity and thus reduced carotenoid synthesis¹⁾. Carotenoids dissipate excess photochemical energy from photosystems through non-photochemical quenching. Insufficient carotenoid concentrations results in unquenched singlet and triplet oxygen destroying chlorophyll molecules; these symptoms are visibly manifested leaf tissue appearing white or bleached.

The first HPPD inhibitor was discovered as an allelopathic chemical produced by the bottlebrush plant (*Callistemon citrinus* Stapf.)⁴⁻⁶⁾. However, leptospermone has low HPPD enzyme inhibitory activity and primarily acropetal movement, and thus has not been commercialized^{5,7)}. It was not until 1982 that from a project screening analogs of the 1,3-cyclohexanedione, acetyl-CoA carboxylase-inhibiting herbicide sethoxydim, that scientists at Zeneca serendipitously discovered a compound that produced bleaching symptoms. Through further structure optimization, this endeavor resulted in the commercialization of the first synthetic triketone HPPD-inhibitor, sulcotrione⁶⁾. This compound provides broadleaf weed control in European corn (*Zea mays* L.) at 300 g ha⁻¹⁸⁻⁹⁾. Continued research and development yielded mesotrione and tembotrione, which are HPPD-inhibitors commonly applied at ≤ 150 and 100 g ha⁻¹, respectively, for post-emergence broadleaf and grassy weed control in U.S. corn markets^{6) 10)}.

The structure-activity relationships of triketone HPPD-inhibiting herbicides are well understood. In their enolic, tautomeric forms, the triketones bind to the non-heme iron group of HPPD, mimicking HPP^{11) 12)}. Therefore, Lee et al.¹⁾ proposed that a 2-benzoyl-ethen-1-ol backbone is the minimum requirement for HPPD inhibition (Figure 2). Extensive research efforts have focused on the contribution of functional groups of both the phenyl and cyclohexane moieties to herbicidal activity and *in vitro* HPPD inhibition.

Investigating phenyl ring substitution patterns, Lee et al.¹³⁾ reported that substituents at the 2,4 and 2,4,5 positions yielded the most active compounds. Further investigation into optimal 2,4 phenyl substituents determined that the 2-nitro-4-methylsulfonyl analog (mesotrione) was most active^{6) 13)}. These phenyl substitutions function largely to increase the acidity, making the molecule more favorable for uptake and translocation in plants and increasing affinity for HPPD *in vitro*^{1) 5)}. Improving on the 2,4-phenyl substitution model, the commercial herbicide tembotrione introduces an alkoxy-trifluoro group at the *meta* position, to likely exploit a lipophilic interaction in the HPPD binding pocket and increase acidity while avoiding intramolecular cyclization^{10) 13) 14)}.

There have been extensive investigations to understand the effect of various substituents on the 1,3-cyclohexanedione moiety. Mitchell et al.⁶⁾ reported that methyl substitutions at the 4 position of the 1,3-cyclohexanedione moiety reduced ED₅₀ (herbicide application rate required to provide 50% control) values against grassy weeds by over 50%. Adding methyl substituents at the chemically equivalent 6 position further reduced ED₅₀ values against grassy weeds. These methyl substitutions did not reduce *in vitro* HPPD IC₅₀ (herbicide concentration required to cause 50% HPPD enzyme inhibition) values. The methyl substituents likely sterically hinder hydroxylation at the 4-position of the cyclohexandione moiety in planta, thus, increasing efficacy

as a result of slower metabolism in target plants⁶⁾. Similarly, Adachi et al.¹⁵⁾ found that adding methyl substituents increased efficacy against grassy weeds but reduced broadleaf weed control. They also found that larger isopropyl or *n*-butyl substitutions greatly reduced herbicidal activity. Similarly, Dayan et al.¹⁴⁾ found bulkier tetraprenyl or tetraethyl cyclohexane ring substituents reduced efficacy. Using HPPD enzyme assays and comparative molecular field analyses, they determined that reduced efficacy of triketones with large cyclohexane substituents is caused by size restrictions in the HPPD binding pocket¹⁴⁾. Despite increased herbicidal activity, these methyl-substituted analogs were not commercialized due to reduced corn tolerance and greater soil persistence⁶⁾. However, in an effort to exploit the potential utility of alkyl-saturated cyclohexandione moieties, Adachi et al.¹⁵⁾ evaluated a bicyclo [4.1.0]-heptane-2,4-dione ring and found efficacy against grassy weeds to be slightly lower than 1,3-cyclohexandione methyl-substituted analogs, but activity against broadleaf weeds was higher. Optimization of the phenyl substituents on this bicycloheptane derivative increased corn tolerance. This technology is utilized by the herbicide bicyclopyrone, which is being developed for weed control in corn, and the pro-herbicide benzobicylon, currently used in rice¹⁶⁾.

Mesotrione is the only HPPD-inhibiting, triketone herbicide registered for use in turfgrass and corn. It is registered for postemergence application at up to 150 g ha⁻¹ in *Zea mays* and the turfgrass *Lolium perenne*, and up to 280 g ha⁻¹ in *Festuca arundinacea* and *Poa pratensis*^{17) 18)}. In *Zea mays*, mesotrione is primarily used to control broadleaf weeds. It can provide control of *Digitaria* and *Echinochloa* species at higher application rates, but it is often applied in combination with an acetolactate synthase inhibiting herbicide to provide annual grassy weed control^{6) 19)}. In turfgrass, many other effective options for broadleaf weed control are available; therefore, mesotrione is used primarily for selective control of grassy weeds such as *Agrostis*

stolonifera, *Poa annua*, and *Digitaria* species. However, complete control of these grassy weeds often requires multiple applications²⁰⁻²²). The efficacy of triketones with alkyl-substituted 1,3-cyclohexanedione moieties against problematic graminaceous weeds of turfgrass has not been evaluated. HPPD-inhibitors with more efficacy and selectivity for certain weedy grasses would have utility in turfgrass management.

The objective of this experiment was to determine how alkyl substitutions on the cyclohexane moiety of triketone herbicides affect control of graminaceous weeds problematic in turfgrass. Our hypothesis was that methyl-substituted analogs would have greater efficacy against grasses such as *Digitaria sanguinalis* and *Eleusine indica*, but not injure desirable cool-season turfgrasses such as *Poa pratensis*.

MATERIALS AND METHODS

Synthesis: General Procedure

The general route used to synthesize 2-[2,4-dichloro]-1,3-cyclohexanediones was similar to that described by Barton et al.²³). The dichloro substitution pattern was selected in favor of stronger electron withdrawing groups to better determine the effects of substitutions at the 4- and 5-positions of the cyclohexanedione moiety. All reactions took place at room temperature in a round-bottom flask with a magnetic stir bar. 1,3-cyclohexanediones and potassium carbonate (Sigma Aldrich, St. Louis, MO, USA) were stirred in excess acetonitrile for 4 hours to generate corresponding enolates. 2,4-dichlorobenzoyl chloride (Sigma Aldrich, St. Louis MO, USA) was dissolved in acetonitrile and added drop wise to the mixture, which was then stirred for one hour until 1,2,4-triazole (Sigma Aldrich, St. Louis, MO, USA) was added. The mixture capped with a rubber stopper, an N₂-filled balloon, and stirred for 24 hours. The acetonitrile was removed by

rotary evaporation under vacuum (Buchi R-114, Buchi Labortechnik AG, Flawil, Switzerland) in 50°C water bath. Water (30 mL) was added and the solution was acidified with sulfuric acid, at which time dichloromethane (30 mL) was added and said mixture was transferred to a fractionation vessel to collect the dichloromethane fraction. This fraction was then dried with magnesium sulfate and filtered through a Büchner funnel connected to an aspirator. The solvent was then removed by rotary evaporation in a room-temperature water bath. If necessary, the resulting crude product was purified using flash chromatography using a gradient elution of ethyl acetate and hexane.

General Analysis

Nuclear magnetic resonance (NMR) spectra of synthetic compounds were obtained using a Varian Gemini spectrometer (Agilent Technologies, Santa Clara, CA, USA) at 300 MHz using CDCl₃ as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in a ppm (δ scale) downfield from TMS. All mass spectrometry analyses were conducted at the Mass Spectrometry Center located in the Department of Chemistry at the University of Tennessee (Knoxville, TN). The analyses were performed by direct measurement in real time (DART) using a JEOL AccuTOF-D time-of-flight (TOF) mass spectrometer with a DART ionization source from JEOL USA, Inc. (Peabody, MA, USA). Mass spectrometry solutions were prepared with CH₂Cl₂.

Compound 1 – 1,3-cyclohexanedione

A total of 0.25 g (2.2 mmol) of 1,3-cyclohexanedione (Sigma Aldrich, St. Louis, MO, USA) and 0.86 g of potassium carbonate (6.25 mmol) were added to acetonitrile (20 mL) and

stirred. After 4 hr, a solution of 0.46 g (2.2 mmol) 2,4-dichlorobenzoyl chloride and 5 mL acetonitrile were added dropwise over a 10 min period to the reaction mixture. After 1 hr, 0.01 g (0.11 mmol) of 1,2,4-triazole was added to the reaction mixture. After 24 hr, the solvent was removed and 30 mL of water was added to the flask. This mixture was acidified with sulfuric acid and 30 mL dichloromethane was added to extract the product. Dichloromethane (30 mL) was added again to the remaining water solution and another extraction was performed and the solvent removed. The product was developed on a thin-layer chromatography (TLC) plate with a 50:50 ethyl acetate:hexane solution. TLC indicated that the product was different from the starting materials and there was only one product to the reaction. Therefore, the product was not subjected to flash chromatography. This reaction yield was 68%, giving 425 mg of an orange, sticky powder. The expected compound 2-(2,4-dichlorobenzoyl)-cyclohexane-1,3-dione was confirmed by DART HRMS m/z (M^+): Calcd. for $C_{13}H_{10}Cl_2O_3$ (m/z): 285.00. Found: 285.01, 287.01, 289.01 in ratios expected from the occurrence of Cl^{35} and Cl^{37} isotopes in a natural 3:1 ratio. 1H NMR δ ($CDCl_3$): 2.14 (2H, q), 2.45-2.47 (2H, m), 2.49-2.71 (2H, m), 6.04 (1H, s), 7.36 (1 H, d), 7.38-7.39 (1H, m), 7.54 (1H, s).

Compound 2 – 4,4-dimethyl-1,3-cyclohexanedione

A total of 0.64 g (4.4 mmol) of 4,4-dimethyl-1,3-cyclohexanedione (Sigma Aldrich, St. Louis, MO, USA) and 0.86 g of potassium carbonate (12.5 mmol) were added to acetonitrile (40 mL) and stirred. After 4 hr, a solution of 0.92 g (4.4 mmol) of 2,4-dichlorobenzoyl chloride and 10 mL of acetonitrile were added dropwise over a 10 min period to the reaction mixture. After 1 hr, 0.015 g (0.22 mmol) of 1,2,4-triazole was added to the reaction mixture. After 24 hr, the solvent was removed and 30 mL of water was added to the flask. This mixture was acidified with

sulfuric acid and 30 mL dichloromethane was added to extract the product. Dichloromethane (30 mL) was added again to the remaining water solution and another extraction was performed and the solvent removed. The product was developed on a thin-layer chromatography (TLC) plate with a 25:75 ethyl acetate:hexane solution. TLC indicated that there were five compounds that differed from starting materials. The compound with the highest R_f value was also present in the highest concentration and was isolated using flash chromatography with a gradient elution of 15 to 35% ethyl acetate/hexane. This reaction yield was 48%, giving 630 mg of a white powder. The expected compound 2-(2,4-dichlorobenzoyl)-4,4-dimethyl-cyclohexane-1,3-dione was confirmed by DART HRMS Calcd. for C₁₅H₁₄Cl₂O₃ (*m/z*): 313.03. Found: 313.04, 315.04, and 317.04 in ratios expected from natural occurrence of Cl³⁵ and Cl³⁷ isotopes in a 3:1 ratio. ¹H NMR δ (CDCl₃): 1.18 (6H, s), 1.94 (2H, t), 2.70 (2H, t), 5.94 (1H, s), 7.37 (1H, dd), 7.53-7.54 (1H, m), 7.90 (1H, d).

Compound 3 – 5-methyl-1,3-cyclohexanedione

A total of 0.83 g (6.6mmol) of 5-methyl-1,3-cyclohexanedione (Alfa Aesar, Ward Hill, MA, USA) and 2.45 g of potassium carbonate (17.75 mmol) were added to acetonitrile (30 mL) and stirred. After 4 hr, a solution of 1.50 g (6.6 mmol) of 2,4-dichlorobenzoyl chloride and 10 mL of acetonitrile were added dropwise over a 10 min period to the reaction mixture. After 1 hr, 0.022 g (0.33 mmol) of 1,2,4-triazole was added to the reaction mixture. After 16 hr, the solvent was removed and 40 mL of water was added to the flask. This mixture was acidified with sulfuric acid and 40 mL dichloromethane was added to extract the product. Dichloromethane (40 mL) was added again to the remaining water solution and another extraction was performed and the solvent removed. The product was developed on a thin-layer chromatography (TLC) plate

with a 25:75 ethyl acetate:hexane solution. TLC indicated that there were two compounds that differed from starting materials with some starting material also remaining. The two compounds with the highest R_f values were present in similar concentrations and were isolated using flash chromatography with a gradient elution with 20 to 40% ethyl acetate/hexane. These two compounds were assumed to be enantiomers. The reaction yield was 28%, giving 550 mg of a beige oil. The expected compound (\pm) 2-(2,4-dichlorobenzoyl)-5-methyl-cyclohexane-1,3-dione was confirmed by DART HRMS m/z (M⁺): Calcd. for C₁₄H₁₂Cl₂O₃ (m/z): 299.01. Found: 299.02, 301.02, and 303.02 in ratios expected from natural occurrence of Cl³⁵ and Cl³⁷ isotopes in a 3:1 ratio. ¹H NMR δ (CDCl₃): 1.15 (6H, s), 1.94 (2H, t), 2.32 (2H, s), 2.56 (2H, s), 6.04 (1H, s) 7.34-7.38 (1H, m), 7.54 (1H, s), 7.90 (1H, d).

Compound 4 – 5,5-dimethyl-1,3-cyclohexanedione

A total of 0.62 g (4.4 mmol) of 5,5-dimethyl-1,3-cyclohexanedione (Sigma Aldrich, St. Louis, MO, USA) and 1.73 g of potassium carbonate (12.5 mmol) were added to acetonitrile (40 mL) and stirred. After 4 hr, a solution of 0.92 g (4.4 mmol) of 2,4-dichlorobenzoyl chloride and 10 mL of acetonitrile were added dropwise over a 10 min period to the reaction mixture. After 1 hr, 0.015 g (0.22 mmol) of 1,2,4-triazole was added to the reaction mixture. After 16 hr, the solvent was removed and 25 mL of water was added to the flask. This mixture was acidified with sulfuric acid and 25 mL of dichloromethane was added to extract the product. Dichloromethane (25 mL) was added again to the remaining water solution and another extraction was performed and the solvent removed. The product was developed on a thin-layer chromatography (TLC) plate with a 25:75 ethyl acetate:hexane solution. TLC indicated that there were two compounds that differed from starting materials. The compound with the highest R_f value was also present in

the highest concentration and was purified using flash chromatography with a gradient elution with 15-35% ethyl acetate/hexane. The reaction yield was 66%, giving 900 mg of an off-white powder. The expected compound 2-(2,4-dichlorobenzoyl)-5-dimethyl-cyclohexane-1,3-dione was confirmed by DART HRMS m/z (M^+): Calcd. for $C_{15}H_{14}Cl_2O_3$ (m/z): 313.03. Found: 313.04, 315.04, and 317.04 in ratios expected from the occurrence of Cl^{35} and Cl^{37} isotopes in a natural 3:1 ratio. 1H NMR δ ($CDCl_3$): 1.15 (6H, s), 1.94 (2H, t), 2.32 (2H, s), 2.56 (2H, s), 6.04 (1H, s) 7.34-7.38 (1H, m), 7.54 (1H, s), 7.90 (1H, d).

Compound 5 – 5-isopropyl-1,3-cyclohexanedione

To acetonitrile (40 mL), 0.68 g (4.4 mmol) of 5-isopropyl-1,3-cyclohexanedione (Alfa Aesar, Ward Hill, MA, USA) and 1.73 g of potassium carbonate (12.5 mmol) were added and stirred. After 4 hr, a solution of 0.92 g (4.4 mmol) of 2,4-dichlorobenzoyl chloride and 10 mL of acetonitrile were added dropwise over a 10 min period to the reaction mixture. After 1 hr, 0.015 g (0.22 mmol) of 1,2,4-triazole was added to the reaction mixture. After 16 hr, the solvent was removed and 40 mL of water was added to the flask. This mixture was acidified with sulfuric acid and 40 mL of dichloromethane was added to extract the product. Dichloromethane (40 mL) was added again to the remaining water solution and another extraction was performed and the solvent removed. The product was developed on a thin-layer chromatography (TLC) plate with a 25:75 ethyl acetate:hexane solution. TLC indicated that there were three compounds that differed from starting materials. The compound with the lowest R_f value was also present in the highest concentration and was purified using flash chromatography with a gradient elution with 32 to 43% ethyl acetate/hexane. The reaction yield was 45%, producing 640 mg of white powder. The expected compound (\pm) 2-(2,4-dichlorobenzoyl)-5-isopropyl-cyclohexane-1,3-dione was

confirmed by DART HRMS m/z (M^+): Calcd. for $C_{16}H_{16}Cl_2O_3$ (m/z): 327.05. Found: 327.05, 329.06, and 331.06 in ratios expected from the occurrence of Cl^{35} and Cl^{37} isotopes in a natural 3:1 ratio. 1H NMR δ ($CDCl_3$): 0.97 (6H, dd), 1.68 (1H, m), 2.19 (1H, m), 2.56 (4H, m), 6.03 (1H, s) 7.36-7.39 (1H, m), 7.54 (1H, d), 7.91 (1H, d).

Compound 6 – 5-phenyl-1,3-cyclohexanedione

A total of 0.42 g (2.2 mmol) of 5-phenyl-1,3-cyclohexanedione (Alfa Aesar, Ward Hill, MA, USA) and 0.86 g of potassium carbonate (6.25 mmol) were added to acetonitrile (40 mL) and stirred. After 4 hr, a solution of 0.46 g (2.2 mmol) of 2,4-dichlorobenzoyl chloride and 5 mL of acetonitrile were added dropwise over a 10 min period to the reaction mixture. After 1 hr, 0.008 g (0.11 mmol) of 1,2,4-triazole was added to the reaction mixture. After 16 hr, the solvent was removed and 40 mL of water was added to the flask. This mixture was acidified with sulfuric acid and 40 mL dichloromethane was added to extract the product. Dichloromethane (40 mL) was added again to the remaining water solution and another extraction was performed and the solvent removed. The product was developed on a thin-layer chromatography (TLC) plate with a 25:75 ethyl acetate:hexane solution. TLC indicated that there were three compounds that differed from starting materials. The compound with the highest R_f value was also present in the highest concentration and was purified using flash chromatography with a gradient elution with 20 to 40% ethyl acetate/hexane. The reaction yield was 53%, producing 420 mg of white powder.

Calcd. for $C_{19}H_{14}Cl_2O_3$ (m/z): 361.03. Found: 361.04, 363.04, 365.04 in ratios expected from the occurrence of Cl^{35} and Cl^{37} isotopes in a natural 3:1 ratio. 1H NMR δ ($CDCl_3$): 1.57 (1H, s), 2.65-3.08 (5H, m), 3.51 (1H, dq), 6.15 (1H, s), 7.30-7.38 (4H, m), 7.54 (1H, d), 7.91 (1H, d).

Compound 7 – 1,3-cycloheptanedione

A solution of 0.55 g (4.4 mmol) of 1,3-cycloheptanedione (Sigma Aldrich, St. Louis, MO, USA) and 5 mL acetonitrile was added dropwise to acetonitrile (50 mL). Then, 1.72 g of potassium carbonate (12.5 mmol) was added and stirred. After 4 hr, a solution of 0.92 g (4.4 mmol) of 2,4-dichlorobenzoyl chloride and 10 mL of acetonitrile were added dropwise over a 10 min period to the reaction mixture. After 1 hr, 0.015 g (0.22 mmol) of 1,2,4-triazole was added to the reaction mixture. After 16 hr, the solvent was removed and 40 mL of water was added to the flask. This mixture was acidified with sulfuric acid and 40 mL of dichloromethane was added to extract the product. Dichloromethane (40 mL) was added again to the remaining water solution and another extraction was performed and the solvent removed. The product was developed on a thin-layer chromatography (TLC) plate with a 25:75 ethyl acetate:hexane solution. TLC indicated that there were two compounds that differed from starting materials. The compound with the highest R_f value was also present in the highest concentration and was purified using flash chromatography with a gradient elution with 20 to 40% ethyl acetate/hexane. The reaction yield was 71%, producing 930 mg of an off-white powder. The expected compound 2-(2,4-dichlorobenzoyl)-cycloheptane-1,3-dione was confirmed by DART HRMS *m/z* (M⁺): Calcd. for C₁₄H₁₂Cl₂O₃ (*m/z*): 299.02. Found: 299.02, 301.02, 303.02 in ratios expected from the occurrence of Cl³⁵ and Cl³⁷ isotopes in a natural 3:1 ratio. ¹H NMR δ (CDCl₃):

Biological Tests

The herbicidal activity of these compounds was assessed by applying them to whole plants grown in a Sequatchie loam soil (fine-loamy, siliceous, semiactive, thermic, and humic Hapludult) having a pH of 5.8 and organic matter content of 2.1% in a glasshouse at the

University of Tennessee (35.98 N, 83.91 W) in 2013. To evaluate postemergence activity, tubers of *Cyperus esculentus* and seeds of *Digitaria sanguinalis*, *Eleusine indica*, *Poa annua*, *Agrostis stolonifera*, *Poa pratensis*, *Stellaria media* and *Trifolium repens* were planted and allowed to grow until they had at least one true leaf prior to by exposed to the previously described compounds. *Digitaria sanguinalis*, *Eleusine indica* and *Stellaria media* were also planted and incorporated in the top 0.5 cm of soil on the day of application to evaluate pre-emergence activity of synthesized compounds. Plants were sowed in six equally spaced, 7.5 cm-long, rows in 10 cm x 30 cm pots. Each of these rows contained a different species. All twelve rows were contained within two pots that were placed adjacent to one another for the duration of the experiment to compose one experimental unit. Pots were treated with a complete (20N-20P₂O₅-20K₂O) fertilizer at 25 kg N ha⁻¹ 24 hours prior to herbicide application.

All compounds were applied at 2500 or 500 g ha⁻¹. Technical-grade mesotrione (Sigma Aldrich, St. Louis, MO, USA) was also included at 2500 or 500 g ha⁻¹ as a commercial standard. Compounds were dissolved in 3 mL acetone and mixed with 14.5 mL of water carrier and crop-oil concentrate (1% v/v) immediately prior to application. This mixture was applied at 460 L ha⁻¹ through an 8002 EVS nozzle (Spraying Systems Co., Wheaton, IL, USA), using a track sprayer (Generation III track sprayer, DeVries Manufacturing, Hollandale, MN, USA).

Treatments were arranged in a completely randomized design with three replications. Visual bleaching/necrosis was assessed for each species individually at 2 weeks after treatment on a 0 (no visual bleaching/necrosis) to 100 (complete bleaching/necrosis) percent scale relative to a non-treated control. These data were subjected to ANOVA ($\alpha \leq 0.05$) and means were separated using Fisher's Protected LSD test.

RESULTS AND DISCUSSION

Herbicide-by-rate interactions were detected for some weed species evaluated; therefore these interactions will be presented for all species tested. Only data collected 2 WAT will be presented, as treatment responses were most apparent at this time.

The 5,5-dimethyl-substituted compound **4** displayed the most activity of any synthesized compounds. At the high (2500 g ha⁻¹) rate, this compound provided > 65% *Eleusine indica* control in both pre- and post-emergence applications. At the low (500 g ha⁻¹) rate, compound **4** provided 63% control of *Eleusine indica* when applied post-emergence but no control from pre-emergence applications. Interestingly, compound **4** provided negligible *Digitaria sanguinalis* control. Given the control provided by the 5,5-dimethyl-substituted compound **4**, previous research suggested that the 4,4-dimethyl-substituted compound **2** would provide similar control⁶. However, compound **2** provided negligible control of all species evaluated. As previous research and our hypothesis suggested, the larger phenyl- and isopropyl-substituted compounds **5** and **6** provided no control^{14) 15)}. None of the compounds synthesized provided any control of *Agrostis stolonifera*, *Poa pratensis*, or *Poa annua*, while mesotrione controlled these grasses > 60%.

With the exception of the *Eleusine indica* control provided by compound **4**, none of the compounds evaluated were as efficacious as mesotrione. However, this was expected as compounds with 2-nitro-4-methylsulfonyl substitutions on the phenyl moiety have an LD₅₀ value more than ten times lower than the 2,4-dichloro substitution pattern on compounds we evaluated¹³⁾.

These results indicate that at least in the case of compound **4**, methyl substitutions on the cyclohexanedione moiety increased control of *Eleusine indica* without causing injury to desirable cool-season turfgrass species such as *Agrostis stolonifera* and *Poa pratensis*. It is possible that *Eleusine indica* relies more heavily on hydroxylation of the cyclohexane ring to

metabolize triketone herbicides than other species. Interestingly, the HPPD-inhibiting herbicide topramezone also has better efficacy than mesotrione against *Eleusine indica* and safety to most cool-season turfgrass species²⁴⁻²⁶). Although topramezone is a pyrazolone and not a triketone compound, it has a methyl group on one of the heteroatoms of its pyrazole ring; this methyl group may serve a similar function as the dimethyl group of compound **4**.

Future research could investigate spiro cyclohexandione analogs such as spiro[2.5]octane-5,7-dione. Other research could investigate compound **4** and analogs for efficacy against populations of *Amaranthus tuberculatus* that are resistant to mesotrione. In some *Amaranthus tuberculatus* populations, resistance is caused by more rapid metabolism of mesotrione to 4-hydroxy-mesotrione, likely a result of cytochrome P450-catalyzed hydroxylation²⁷). Methyl-saturated compounds similar to those evaluated in this experiment would be less susceptible to hydroxylation could be used to diagnose and control resistant populations.

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APPENDIX
TABLES AND FIGURES

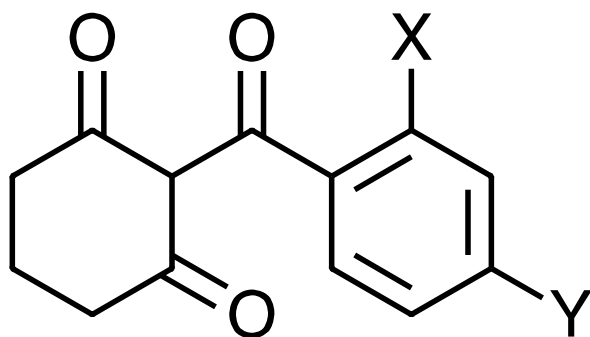


Figure 4.1. Generic structure of the 2-benzoylcyclohexane-1,3-dione herbicides.

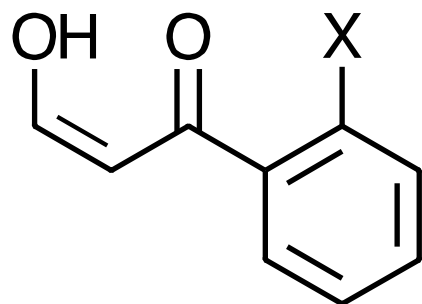
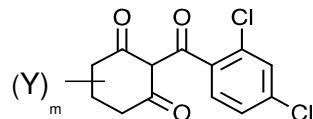


Figure 4.2. Minimum structural requirement for HPPD inhibitors proposed by Lee et al. (1997).

Table 4.1. Visual bleaching 14 days after application of 2-(2,4-dichlorobenzoyl)cyclohexane-1,3-dione derivatives and compound **7**, 2-(2,4-dichlorobenzoyl)cycloheptane-1,3-dione. Compounds were applied at 2500 and 500 g ha⁻¹. Compounds were dissolved in 3 mL acetone and mixed with 14.5 mL of water carrier and crop-oil concentrate (1% v/v) immediately prior to application.



No.	(Y) _m	Rate	DIGSA ^a	ELEIN	AGSST	POAPR	POAAN	CYPES	TRIRe	STEME	DIGSA (PRE)	ELEIN (PRE)	STEME (PRE)
		g ha ⁻¹						% Visual Bleaching and Necrosis					
1	-	2500	0	0	0	0	0	7	7	0	17	0	0
		500	13	0	0	0	0	23	0	3	0	0	0
2	4,4-(CH ₃) ₂	2500	3	7	0	0	0	0	0	0	0	0	0
		500	3	0	0	0	0	7	0	0	0	0	0
3	5-(CH ₃)	2500	16	0	0	0	0	17	7	0	0	0	0
		500	0	0	0	0	0	5	0	0	0	0	0
4	5,5-(CH ₃) ₂	2500	27	80	0	0	0	65	13	17	13	67	0
		500	13	63	0	0	0	13	0	3	0	7	0
5	5-C ₃ H ₇ ⁱ	2500	7	0	0	0	0	0	7	0	0	0	0
		500	0	0	0	0	0	0	0	0	0	0	0
6	5-C ₆ H ₅ ^{Ph}	2500	7	0	0	0	0	0	0	0	0	0	0
		500	3	0	0	0	0	0	0	0	0	0	0
7	-	2500	0	0	0	0	0	0	0	0	0	0	0
		500	7	0	0	0	0	13	0	7	0	0	0
	Mesotrione	2500	67	92	90	78	80	95	97	77	100	100	97
		500	67	90	83	63	67	93	93	70	100	100	93
	LSD _{0.05} ^b		39	11	5	16	13	25	11	11	13	25	3

^aAbbreviations: DIGSA: *Digitaria sanguinalis*, ELEIN: *Eleusine indica*, AGSST: *Agrostis stolonifera*, POAPR: *Poa pratensis*, POAAN: *Poa annua*, CYPES: *Cyperus esculentus*, TRIRe: *Trifolium repens*, STEME: *Stellaria media*, PRE: Indicates control of species that was planted from seed on the day of herbicide application.

^bIndicates Fisher's Protected LSD ($P \leq 0.05$)

CONCLUSIONS

New herbicides are developed and optimized for selectivity in corn, wheat and soybeans but not turfgrass. As of this writing, it is expected that few new herbicides will be released to the turfgrass market in the next several years. Especially with the emergence of weed resistance in turfgrass systems, increasing the number of herbicide options for weed control is important. This research explores strategies that can be used to find new uses for herbicides already in the marketplace.

One strategy involved synthesizing and evaluating molecular analogs of the triketone herbicide mesotrione that was developed for weed control in corn. This research determined that one triketone analog provided excellent goosegrass control and did not injure creeping bentgrass.

Another strategy that has received limited investigation in turfgrass, uses compounds described as herbicide safeners to increase tolerance of desirable plants to a herbicide. The herbicide topramezone can cause injury to creeping bentgrass at rates that effectively control weeds and thus applications are prohibited. Several experiments determined that the herbicide safener cloquintocet-mexyl can increase creeping bentgrass tolerance to topramezone without reducing control of large crabgrass or goosegrass. Additional research demonstrated that creeping bentgrass tolerance to topramezone is influenced by cytochrome P450 monooxygenases. This is one mechanism by which herbicide safeners may increase creeping bentgrass tolerance to topramezone. Other experiments determined that the herbicide safeners cloquintocet-mexyl and mefenpyr-diethyl can reduce pinoxaden injury to creeping bentgrass. These safeners did not reduce pinoxaden efficacy against perennial ryegrass, but slightly reduced efficacy against roughstalk bluegrass. Field research is needed to develop topramezone and pinoxaden in combination with herbicide safeners for use in creeping bentgrass.

VITA

Matthew Thomas Elmore was born on July 6, 1987 in Columbia S.C. to Donna and Jim Elmore. Raised in Lugoff, S.C., he eventually relocated with his family to Lincoln University, PA at the age of 5. He attended St. Mark's High School in nearby Wilmington, DE and graduated in 2005. Enrolling in the Turfgrass Science program at Penn State University, he graduated in 2009 with a B.S. degree. He graduated with an M.S. degree from the University of Tennessee in 2011. Matt plans work as an Assistant Professor and Turfgrass Extension Specialist at Texas A&M University in Dallas, Texas after graduation.