Optimizing Topramezone and Other Herbicide Programs for Weed Control in Bermudagrass and Creeping Bentgrass Turf

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Academic Abstract

Goosegrass [Eleusine indica (L.) Gaertn.] and smooth crabgrass [Digitaria ischaemum (Schreb.) Schreb. ex Muhl.] are problematic weeds in bermudagrass and creeping bentgrass turf. Increased incidences of herbicide resistant weed populations and severe use restrictions on formerly available herbicides have increased need for selective, postemergence control options for these weeds in creeping bentgrass and bermudagrass turf. This weed management exigency has led turf managers to utilize less effective, more expensive, and more injurious options to manage goosegrass and smooth crabgrass. Although potentially injurious, topramezone can control these weeds, especially goosegrass, at low doses. Low-dose topramezone may also improve bermudagrass and creeping bentgrass response.

An initial investigation of three 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibiting herbicides in different turf types showed that Kentucky bluegrass, perennial ryegrass, and tall fescue were highly tolerant to topramezone, while creeping bentgrass and bermudagrass could tolerate topramezone doses that may control grassy weeds. Further investigation suggested that frequent, low-dose topramezone applications or metribuzin admixtures could enhance weed control and may conserve turfgrass quality. A novel mixture of topramezone at 3.7 g ae ha⁻¹ and metribuzin at 210 g ai ha⁻¹ controlled goosegrass effectively and reduced bermudagrass foliar bleaching associated with topramezone 10-fold compared to higher doses of topramezone alone in 19 field and 2 greenhouse trials. In an attempt to further enhance bermudagrass tolerance to topramezone, post-treatment irrigation was applied at various timings. When bermudagrass turf was irrigated with 0.25-cm water at 15 or 30 minutes after herbicide treatment, bermudagrass injury was reduced to acceptable levels when following low-dose topramezone plus metribuzin but not when following high-dose topramezone alone. Goosegrass control was reduced significantly by post-treatment irrigation in all cases, while irrigation reduced goosegrass control by low-dose topramezone plus metribuzin to below-commercially-acceptable levels. Novel, low-dose, frequent application programs containing topramezone or siduron were developed for season-long crabgrass or goosegrass control on creeping bentgrass greens. Greens-height creeping bentgrass quality was preserved following five biweekly treatments of siduron at rates between 3,400 to 13,500 g ai ha⁻¹ and topramezone at 3.1 g ha⁻¹. Siduron programs controlled smooth crabgrass and suppressed goosegrass while topramezone programs controlled goosegrass and suppressed smooth crabgrass.

In laboratory and controlled-environment experiments, goosegrass absorbed three times more ¹⁴C than bermudagrass within 48 hours of ¹⁴C-topramezone treatment. Bermudagrass also metabolized topramezone twice as fast as goosegrass. Metribuzin admixture reduced absorption by 25% in both species. When herbicides were placed exclusively on soil, foliage, or soil plus foliage, topramezone controlled goosegrass only when applied to foliage and phytotoxicity of both bermudagrass and goosegrass was greater from topramezone than from metribuzin. Metribuzin was shown to reduce 21-d cumulative clipping weight and tiller production of both species while topramezone caused foliar discoloration to newly emerging leaves and shoots with only marginal clipping weight reduction. These data suggest that selectivity between bermudagrass and goosegrass is largely due to differential absorption and metabolism that

reduces bermudagrass exposure to topramezone. Post-treatment irrigation likely reduces topramezone rate load with a concomitant effect on plant phytotoxicity of both species. Metribuzin admixture decreases white discoloration of bermudagrass by decreased topramezone absorption rate and eliminating new foliar growth that is more susceptible to discoloration by topramezone.

Optimizing Topramezone and Other Herbicide Programs for Weed Control in Bermudagrass and Creeping Bentgrass Turf

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General Audience Abstract

Goosegrass and smooth crabgrass are problematic weeds in bermudagrass and creeping bentgrass turf. Increased incidences of herbicide resistant weed populations and severe use restrictions on formerly available herbicides have increased need for selective, postemergence control options for these weeds in creeping bentgrass and bermudagrass turf. Although potentially injurious, topramezone (PylexTM) can control these weeds, especially goosegrass, at low doses. Low-dose PylexTM may also improve bermudagrass and creeping bentgrass response.

An initial investigation evaluating tembotrione (Laudis®), PylexTM, and mesotrione (Tenacity®) in different turfgrass species showed that Kentucky bluegrass, perennial ryegrass, and tall fescue were highly tolerant to PylexTM at rates ranging from 0.75 to 2.25 fl. oz./A, while creeping bentgrass and bermudagrass were low to moderately tolerant to PylexTM. Further investigation suggested that frequent, low-dose (less than 0.25 fl. oz./A) PylexTM applications or metribuzin (Sencor®) admixtures could enhance weed control and may conserve turfgrass quality. A novel mixture of PylexTM at 0.15 fl. oz./A and Sencor® at 4 oz. wt./A controlled goosegrass effectively and reduced bermudagrass injury to near acceptable levels and significantly less than PylexTM applied alone at 0.25 fl. oz/A. In an attempt to further enhance bermudagrass tolerance to PylexTM, post-treatment irrigation was applied at different timings. When bermudagrass turf was irrigated at 15 or 30 minutes after herbicide treatment, bermudagrass injury was reduced to acceptable levels when following PylexTM at 0.25 fl. oz./A plus Sencor® at 4 oz but not when following PylexTM applied alone at 0.5 fl. oz./A. Goosegrass control was reduced significantly by post-treatment irrigation in all cases, while irrigation reduced goosegrass control by low-dose PylexTM plus Sencor® to below-commerciallyacceptable levels. Novel, low-dose, frequent application programs containing PylexTM or siduron (Tupersan®) were developed for season-long crabgrass or goosegrass control in creeping bentgrass greens. Greens-height creeping bentgrass quality was preserved following five biweekly treatments of Tupersan® at rates between 6 and 24 lb./A and PylexTM at 0.125 fl. oz./A. Tupersan® programs controlled smooth crabgrass and suppressed goosegrass while PylexTM programs controlled goosegrass and suppressed smooth crabgrass.

The data from these studies indicate that utilizing low-dose PylexTM in combination with Sencor® can impart acceptable bermudagrass safety while also controlling goosegrass effectively. For creeping bentgrass greens, the low-dose, frequent application of Tupersan® is the safest legal option for golf course superintendents to control smooth crabgrass effectively, while having some ability to suppress goosegrass.

I dedicate this dissertation to all of my family and friends who have supported me during my
years at graduate school, especially my parents, Richie and Nina, and my sister Kaylea and
brother-in-law Tyler, for their constant unwavering love and support.

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ATTRIBUTIONS

Chapter II. Response of Six Turfgrass Species and Four Weeds to Three HPPD-Inhibiting Herbicides.

John Brewer, Ph.D. candidate in Turfgrass Weed Science in the School of Plant and Environmental Sciences at Virginia Tech. He assisted in visual and digital data collection and interpretation for the field studies, as well as the main author for this chapter.

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Chapter III. Bermudagrass and Goosegrass Response to Low-rate Topramezone and Metribuzin Programs.

John Brewer, Ph.D. candidate in Turfgrass Weed Science in the School of Plant and Environmental Sciences at Virginia Tech. He assisted in formulating the experimental and treatment designs for the field, greenhouse, and laboratory studies. He initiated and conducted the studies which included the management of the study sites as well as collection and interpretation of visual and digital data for all studies. He is also the main author for this chapter. Shawn D. Askew, Ph.D., is a professor of Turfgrass Weed Science in the School of Plant and Environmental Sciences at Virginia Tech. He assisted with formulating the experimental and treatment design of the field, greenhouse, laboratory studies, as well as statistical data analyses.

Whitnee Askew, M.S., is the Turfgrass Program Manager for the School of Plant and Environmental Sciences at Virginia Tech. She assisted in the protocol and methods development as well as data collection for the ¹⁴C-topramezone absorption, translocation, and metabolism experiment.

Chapter IV. Influence of Post-Treatment Irrigation Timings on Bermudagrass and Goosegrass Response to Low-Rate Topramezone Plus Metribuzin Programs.

John Brewer, Ph.D. candidate in Turfgrass Weed Science in the School of Plant and Environmental Sciences at Virginia Tech. He assisted in formulating the experimental and treatment designs for the field and greenhouse studies. He also initiated and conducted the studies which included the management of the study sites and collection and interpretation of all visual and digital data. He is also the main author for this chapter.

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Chapter V. Investigating Topramezone Rates and Other Herbicide Programs for Weed Control on Creeping Bentgrass Greens.

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Chapter IV. Influence of Post-Treatment Irrigation Timings on Bermudagrass and Goosegrass Response to Low-Rate Topramezone Plus Metribuzin Programs.

-None

Chapter V. Investigating Low-Dose Herbicide Programs for Goosegrass (*Eleusine indica*) and Smooth Crabgrass (*Digitaria ischaemum*) Control on Creeping Bentgrass Greens

-None

Chapter 1. Literature Review

Goosegrass [Eleusine indica (L.) Gaertn.] and smooth crabgrass [Digitaria ischaemum (Schreb.) Schreb. ex Muhl.] have progressively become two of the most challenging and problematic weeds to manage in both bermudagrass [Cynodon dactylon (L.) Pers.] and creeping bentgrass (Agrostis stolonifera L.) turfs. Goosegrass is ranked as one of the top five most troublesome weeds worldwide as well as one of the three most problematic weeds on golf courses in the southeastern United States (Anonymous 1996; Holm et al. 1991). Similarly, smooth crabgrass is also considered by many to be one of the most common and competitive weeds to manage in most turfgrass swards (Bhowmik 1987; Dernoeden et al. 1993a; Kim et al. 2002). The commonality and competitiveness of the two grasses can cause major issues in uniformity and quality of the turfgrass canopy which decreases aesthetics and playability in some settings (Dernoeden et al. 1993a; Rana and Askew 2018; SportsTurf 2016). Decreases in playability due to these weeds and others occurs on athletic fields by causing poor footing/tripping hazards for players and also on golf courses, specifically putting greens, weeds can cause golf balls to be diverted and miss their target (Rana and Askew 2018; SportsTurf 2016).

Goosegrass thrives as a competitor with desirable turfgrasses in compacted soils that receive heavy foot and vehicular traffic since its better adapted to survive in low oxygen environments (Beard 2002; McCarty et al. 2005; Waddington and Baker 1965). Both goosegrass and smooth crabgrass are opportunistic invaders that germinate rapidly and grow aggressively through thin turf canopies or bare areas, situations frequently observed on the most heavily trafficked zones of golf and sports turfs, while also surviving hot and dry environments (Beard 2002; McCarty et al. 2005). These grasses have high fecundity and can produce over 140,000

seed per plant per year (Chin 1979; Mitich 1988; Peters and Dunn 1971; Xiao-yan et al. 2015). The high number of potential progeny and the ability for them to germinate continuously from late spring to late summer can cause the management strategies for goosegrass and smooth crabgrass to be more difficult to plan from year to year (Fidanza et al. 1996; Kerr et al. 2018). Even when both grasses are effectively managed from year to year, their seed can survive for at least three years with goosegrass seed surviving greater than three years (Masin et al. 2006).

Multiple other factors could increase both the difficulty in managing goosegrass and smooth crabgrass in turf and their prevalence in the previously described areas. In the past few years, turf managers have observed a general increase in management cost while also having a decrease in budgets at many turf facilities (USGA 2020). This may reduce the use of fertilizers, plant protectants, and general cultural practices in order to cut annual maintenance costs, which, in turn, can decrease turf vigor and increase the potential for weed encroachment (Dernoeden et al. 1993a).

Other issues that have begun to plague the turfgrass industry include use restrictions and/or complete loss of effective herbicide options for goosegrass and smooth crabgrass in both the cool-season and warm-season turfgrass markets. There are multiple pre-emergent herbicides that can control goosegrass and smooth crabgrass effectively including dithiopyr, prodiamine, and oxadiazon. However, many turf sectors, especially golf courses, are beginning to have increases in goosegrass populations resistant to herbicides with the following modes of action: microtubule inhibitors (prodiamine, pendimethalin, trifluralin), protoporphyringogen oxidase inhibitors (oxadiazon), and photosystem II inhibitors (metribuzin) (Breeden et al. 2017; Brosnan et al 2008; McCullough et al. 2013; McElroy et al. 2017; Mudge et al. 1984; Heap 2020). At this time, herbicide resistance has not yet become a major factor for smooth crabgrass management,

but that issue is on the horizon as we see resistant populations in other cropping systems and anecdotal evidence of populations that already exist in turf (Brosnan et al. 2020; Heap 2020). In bermudagrass and creeping bentgrass, effective management options for these grasses may be limited due to the lack of effective herbicides, budget constraints, and increased resistance issues.

Goosegrass and Smooth Crabgrass Management in Bermudagrass and Creeping Bentgrass

Both the combination of monosodium acid methanearsonate (MSMA) plus metribuzin and diclofop are proven treatments for goosegrass control in bermudagrass. Busey et al. (2004) observed that MSMA plus metribuzin controlled mature goosegrass approximately 75 to 100% after two applications, while MSMA applied alone controlled goosegrass 42% or less. Other studies observed less goosegrass control than Busey et al. (2004), which may be due to single applications instead of two applications (McCarty 1991). Johnson (1980) observed similar results from single and double applications of MSMA plus metribuzin for goosegrass control as Busey et al. (2004) and McCarty (1991). For many years, MSMA was also the most common herbicide for crabgrass species control. Monosodium acid methanearsonate is inconsistent for controlling large crabgrass [Digitaria sanguinalis (L.) Scop.] and smooth crabgrass when applied only once at 2.2 kg ai ha⁻¹ (Johnson 1975, 1993, 1994a, 1996). Researchers have observed that MSMA applied once at 2.2 kg ai ha⁻¹ controlled large crabgrass from 10 to 99% (Johnson 1975, 1994a, 1996) and smooth crabgrass from 49 to 88% (Derr 2002; Neal et al. 1990). When applied twice every seven to ten days, MSMA applied at 2.2 kg ai ha⁻¹ controlled large crabgrass 40 to 100% and was more effective then single applications (Johnson 1975, 1996). Metribuzin applied at 0.14 kg ai ha⁻¹ plus MSMA applied at 2.2 kg ai ha⁻¹ controlled large crabgrass effectively from 70 to 91% (Johnson 1994c). MSMA applied alone or in combination with metribuzin injured bermudagrass between 30 to 40% during multiple research studies (Johnson 1993, 1994c).

Another herbicide that was an effective option for goosegrass control was the graminicide (acetyl CoA carboxylase inhibitor or ACCase inhibitor) diclofop. Researchers observed that diclofop controlled goosegrass greater than 90% after June applications, and controlled goosegrass from 80 to 90% when treated in August (McCarty 1991). When goosegrass has matured and is mown at or above 4 cm, researchers have observed that diclofop applied alone controlled goosegrass less than 30% (Nishimoto and Murdoch 1999). Diclofop also caused little to no injury to bermudagrass (McCarty 1991). Even though it's an effective option, diclofop is no longer manufactured.

Due to heavy restrictions to MSMA use in turfgrass by the Environmental Protection Agency (EPA) and loss of diclofop availability, the programs that utilized these herbicides for goosegrass control in bermudagrass are no longer viable treatment options (Keigwin 2013; McCullough 2014). The restrictions and/or loss of these herbicides has led bermudagrass turf managers to rely on treatments that are more expensive and more injurious to turf, such as foramsulfuron-containing products and programs with metribuzin and sulfentrazone combinations. Researchers observed that foramsulfuron-containing programs controlled goosegrass effectively when it was applied sequentially to goosegrass at less than three-tiller maturity stage, or when foramsulfuron is applied in combination with metribuzin to goosegrass larger than three-tiller stage (Busey 2004). Unfortunately, these multi-application programs are cost prohibitive for many turfgrass managers if the programs consist of two or more applications (Anonymous 2020). Metribuzin applied at rates greater than 280 g ai ha⁻¹ controlled goosegrass effectively, but these metribuzin programs also caused unacceptable bermudagrass turf quality (Busey 2004; Johnson 1975; Johnson 1980). McCullough et al. (2012) observed that sulfentrazone controlled mature goosegrass from 30 to 65%, but can have higher control levels if applied to seedling goosegrass (Anonymous 2008). Sulfentrazone can also significantly injure turfgrass when using high rates during stressful environmental periods (McCullough et al. 2014). Other acetolactate synthase (ALS) inhibitors similar to foramsulfuron like trifloxysulfuron and flazasulfuron can be utilized for crabgrass species control in bermudagrass as well, but these are not used regularly since cheaper and more effective options exist (Anonymous 2021a, 2021b; Johnson 1995; Willis et al. 2007). Flazasulfuron applied at 35 g ai ha⁻¹ and trifloxysulfuron applied at 29 g ai ha⁻¹ controlled seedling smooth crabgrass 96 and 92%, respectively (Willis et al. 2007). Foramsulfuron did not control smooth crabgrass effectively (Willis et al. 2007).

Since the introduction of quinclorac in 1999 followed by the MSMA restrictions in 2013, quinclorac has become the standard herbicide for post-emergent control of crabgrass species (Kiegwin 2013; Beard 1999). Quinclorac applied once at 0.84 kg ai ha⁻¹ could have variable control of crabgrass similar to MSMA. Multiple researchers have observed that quinclorac controlled large crabgrass 80 to 100% and smooth crabgrass from 20 to 95% (Derr 2002; Dernoeden et al. 2003; Johnson 1993, 1994b, 1996). Quinclorac controlled smooth crabgrass more consistently and effectively when applied twice at 0.56 kg ai ha⁻¹ or three times at 0.42 kg ai ha⁻¹ (40 to 99% and 92 to 99%, respectively) (Dernoeden et al. 2003). Quinclorac-based programs can injure bermudagrass between 30 to 40% which is typically classified as unacceptable (Dernoeden et al. 2003; Johnson 1993, 1996). Recently, a new ACCase-inhibitor called pinoxaden was labeled in 2018 for use in bermudagrass to control graminaceous weeds including crabgrass species (Anonymous 2018b). It is an effective option for crabgrass control but it is recommended to be applied to seedling crabgrass or applied at high rates as crabgrass matures (Peppers et al. 2020; Anonymous 2018b). Pinoxaden is relatively ineffective for goosegrass control (Peppers et al. 2020).

For creeping bentgrass areas, herbicide resistance and herbicide use restrictions are as equally problematic for goosegrass and smooth crabgrass management as in bermudagrass. This is mainly due to creeping bentgrass sensitivity to many effective grass-control products like fenoxaprop and quinclorac which must be labeled at lower and less effective rates compared to many other turf species (Anonymous 2013, 2019). Fenoxaprop can be an effective option for goosegrass and smooth crabgrass control, but the rate registered for creeping bentgrass is 8 to 10 times lower than all other cool-season species, and it must be applied when the weedy grasses are less than two-leaf stage (Anonymous 2013). Even then, you need multiple applications to achieve significant control (Anonymous 2013; Leibhart et al. 2014). Fenoxaprop can also injure creeping bentgrass unacceptably as rate and application number increase (Carroll et al. 1992; Henry and Hart 2004; Shim and Johnson 1992). Another herbicide that can control seedling goosegrass and smooth crabgrass is sulfentrazone, but control rapidly declines as these species mature (Anonymous 2008; Leibhart et al. 2014). Researchers have also observed that Speedzone® (a broadleaf herbicide containing carfentrazone, dicamba, 2,4-D, and MCPP), controlled seedling to multi-tiller goosegrass between 50 and 80%, but it has to be applied at 4.68 L ha⁻¹ with two to three total applications two weeks apart to be an effective option (Leibhart et al. 2014). Unfortunately, Speedzone® is only labeled to be applied twice at a 30-day reapplication interval (Anonymous 2018c). Interestingly, for smooth crabgrass control in creeping bentgrass areas mown above 0.6 cm, most managers rely on the previously mentioned pre-emergent options or quinclorac. Quinclorac is an effective post-emergent herbicide for young to mature smooth crabgrass, but it may take multiple applications at the labeled bentgrass rate to achieve acceptable control (Dernoeden et al. 2003; Hart et al. 2004; Johnson 1994b). Unfortunately, it has relatively low activity on goosegrass at all growth stages (Johnson 1994b).

Quinclorac applied to creeping bentgrass mown below 0.5 cm or newly established stands can cause significant and unacceptable phytotoxicity (Dernoeden et al. 2003; Hart et al. 2004).

When looking for management programs in green-height (less than 0.5 cm) creeping bentgrass, almost all options for goosegrass and smooth crabgrass control are nonexistent. Only the pre-emergent herbicides bensulide, dithiopyr, oxadiazon, and siduron are labeled for goosegrass and smooth crabgrass control on creeping bentgrass greens (Anonymous 2014, 2016, 2017, 2018). When these four pre-emergent herbicides are compared for goosegrass and crabgrass species control, all four herbicides control crabgrass effectively, while oxadiazon is the best for goosegrass control followed closely by dithiopyr (Callahan 1986; Hart et al. 2004; Johnson 1993, 1994). Even though these pre-emergent options can be effective for weed control, many managers are hesitant to utilize them due to the potential for unacceptable turf injury and turf loss. Siduron is the safest of the four pre-emergent herbicides, and rarely causes unacceptable phytotoxicity. Dithiopyr and bensulide can cause unacceptable turf and root mass losses when applied in late summer to early fall, while very little injury occurs to creeping bentgrass when they are applied in the spring (Bingham and Schmidt 1983; Callahan 1972; Dernoeden et al. 1993b; Hart et al. 2004; Shim and Johnson 1992). Bensulide and oxadiazon can cause unacceptable phytotoxicity if applied multiple times or during heat and drought stress in mid to late summer (Callahan 1972; Dernoeden et al.1993b). Even when pre-emergent products are used appropriately, many managers still have crabgrass and goosegrass emergence on their greens.

Currently, there is only one registered post-emergent herbicide for control of crabgrass spp., which is a product that contains 2,4-D, dicamba, and quinclorac and is called 2DQ® (Adama: Control Solutions Inc., 5903 Genoa-Red Bluff, Pasadena, TX 77507). The rate of

quinclorac utilized by this product on creeping bentgrass greens is eight times lower than rates previously researched on creeping bentgrass, and there is no research supporting the use of this product for bentgrass safety or crabgrass control (Anonymous 2019; Dernoeden et al. 2003). At this time, there are still no post-emergent herbicide options available for goosegrass control on creeping bentgrass greens; mechanical removal with a knife is often employed. Due to the time and expense with mechanical removal, some managers experiment with risky and unlabeled chemical control options such as fenoxaprop and different quinclorac-containing products.

Researchers have evaluated low rate programs of both herbicides for use on green height turf and found that unacceptable phytotoxicity to bentgrass can occur. Fenoxaprop applied at 0.04 kg ha⁻¹ (a level three times lower than most cool-season turfgrass application rates) reduced 'Penn A-4' creeping bentgrass turf quality to an unacceptable level (Henry and Hart 2004). Carroll et al. (1992) also observed fenoxaprop applied consecutively between 0.03 and 0.05 kg ha⁻¹ injuring creeping bentgrass significantly. Quinclorac has also been observed to injure creeping bentgrass greens unacceptably when applied at rates as low as 0.6 kg ha⁻¹ (Johnson 1994a).

Metamifop is another herbicide option for creeping bentgrass greens due to its ability to control goosegrass and smooth crabgrass effectively and it is safe to the turf. Unfortunately, it is not labeled in turfgrass at this time. Researchers have observed metamifop controlling two to three tiller goosegrass and smooth crabgrass effectively when applied between 200 to 400 g ai ha⁻¹ (Cox and Askew 2014; Parker et al. 2015). Sequential applications of metamifop applied from 100 to 200 g ai ha⁻¹ have also controlled both weed species effectively, and may be necessary as the weeds increase in size and maturity (Cox and Askew 2014; Parker et al. 2015). Metamifop has been safely applied to mature creeping bentgrass at rates as high as 800 g ai ha⁻¹, but other researchers observed metamifop unacceptably injuring 12-week-old creeping bentgrass

at rates as low as 300 g ai ha⁻¹ (Cooper et al. 2017; Parker et al. 2015). These data indicate that future research should focus on metamifop applied sequentially between 100 and 200 g ai ha⁻¹ for green-height turf.

Topramezone- New Herbicide for Turfgrass

A new 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide from the pyrazolone family called topramezone was recently labeled in both creeping bentgrass (2015) and bermudagrass (2018) turf primarily for controlling goosegrass (Pylex label; herbicide handbook). It is an effective broad-spectrum herbicide that was first labeled in corn (Zea mays L.) to control or suppress more than 40 weed species (Bollman et al. 2008; Soltani et al. 2012). When susceptible plants are treated, topramezone is readily and rapidly absorbed by the leaves and roots, and it is translocated systemically through the phloem both acropetally and basipetally (Grossman and Ehrhardt 2007). In both tolerant and susceptible species, 60% of topramezone is absorbed in less than 10 hours and 80% by 24 hours after treatment, which reveals absorption levels or rates are not the reason for tolerance in many species. Tolerant species like corn rapidly metabolize topramezone to topramezone-desmethyl (50 times lower herbicide activity than topramezone) and other metabolites, while also being less sensitive to the 4-HPPD enzyme (Grossman and Ehrhardt 2007). It is important to note that topramezone has a low water solubility (0.69 mg L⁻¹) and needs a compatible surfactant to be properly absorbed by susceptible weed species (Grossman and Ehrhardt 2007; Senseman 2007; Zhang et al. 2013). Topramezone can also be absorbed effectively from moderate to high temperatures, low to high light intensities, and low to high humidity (Grossman and Ehrhardt 2007).

Topramezone controls weeds by inhibiting the 4-HPPD enzyme. By blocking the HPPD enzyme, topramezone inhibits the biosynthesis of prenylquinones, plastoquinone, and

tocopherol. Since plastoquinone is thought to be an important cofactor to phytoene desaturase, it indirectly blocks or slows the production of important carotenoids such as ζ -carotene, lutein, violaxanthin, and zeaxanthin (Boger et al. 1998; Norris et al. 1995; Sandmann 2000). The reduction of carotenoid production removes plant defensive measures against reactive oxygen species that causes photooxidation. Since carotenoids are no longer available to quench tripletand singlet-state oxygen nor dissipate the excess energy as heat through nonphotochemical quenching, oxidative degradation of chlorophyll begins within the new shoots as well as lipid peroxidation of other important organelles that leads to bleached plant tissue, then necrosis, and finally plant death (Boger et al. 1998; Demmig-Adams and Adams 1996; Demmig-Adams et al. 1996; Niyogi 1999; Sandmann 2000). The loss of tocopherol production also adds to the decrease in buffering capacity to the reactive oxygen species which will also increase the rate of plant death (Matringe et al. 2005). Topramezone and many HPPD-inhibiting herbicides are increasingly utilized every year in many cropping systems, including turf, because of their effectiveness on many troublesome weed species such as herbicide resistant Palmer amaranth (Amaranthus palmeri S. Watson) and goosegrass (Breeden et al. 2017; Kohrt and Sprague 2017).

Topramezone: Turf Tolerance and Annual Grass Control

In creeping bentgrass, topramezone is labeled at 6.1 g ai ha⁻¹, while it is labeled at 12.3 to 18.4 g ai ha⁻¹ in bermudagrass. Those rates are approximately one-sixth to one-half the labeled rates in other cool-season species. Even at these lower rates, both bermudagrass and creeping bentgrass are susceptible to unacceptable injury and foliar bleaching, but for both species the injury is transient and full recovery occurs three to five weeks after application (Brewer et al. 2016; Brosnan et al. 2011; Cox et al. 2017; Elmore et al. 2011). Topramezone typically injures creeping bentgrass two to three times less than bermudagrass at similar rates (Brewer et al. 2016;

Brosnan et al. 2011; Elmore et al. 2015b). Elmore et al. (2015a) observed that more rapid topramezone metabolism by creeping bentgrass may be the reason for differential safety to topramezone compared to susceptible weed species. Metabolism may also help explain why creeping bentgrass is less affected by topramezone than bermudagrass, but no studies have evaluated topramezone metabolism in bermudagrass compared to other sensitive species like goosegrass. Researchers observed that topramezone applied from 18.4 to 55.2 g ai ha⁻¹ injured creeping bentgrass an average of 18 to 30% AUPC day⁻¹ while the same rate injured bermudagrass an average of 60 to 70% AUPC day⁻¹ (Brewer et al. 2016). In another study, topramezone applied at 37 g ai ha⁻¹ caused a maximum of 27% bleaching on creeping bentgrass, while other researchers observed the same rate cause 50% bleaching or greater on bermudagrass (Brosnan et al. 2011; Elmore et al. 2015b).

Due to creeping bentgrass and bermudagrass sensitivity to topramezone, many researchers have begun to assess different ways to reduce foliar bleaching and general injury, while maintaining goosegrass control. These include tank mixing different fertility products, herbicides, and safeners with topramezone in conjunction with lower rate regimes. Elmore et al. (2015a&b) observed cloquintocet-mexyl applied at rates equal to or greater than 28 g ai ha⁻¹ reduced topramezone-induced creeping bentgrass injury from 23% to 11% without reducing goosegrass and smooth crabgrass control. At Virginia Tech, researchers have anecdotal evidence that the addition of triclopyr applied between 17.5 to 35.1 g ae ha⁻¹ can effectively reduce the foliar bleaching on creeping bentgrass caused by topramezone applied at 6.13 to 12.3 g ai ha⁻¹. Due to the results observed in the creeping bentgrass research at Virginia Tech, multiple researchers have assessed tank-mixing triclopyr at 140 g ae ha⁻¹ with topramezone at 6.13 or 12.3 g ai ha⁻¹ in bermudagrass to determine if the combination could reduce foliar bleaching

effectively without any negative side effects. Across two separate studies, triclopyr effectively reduced the bleaching caused by topramezone but it increased bermudagrass necrosis and duration of the injury to an unacceptable level (Boyd et al. 2020; Cox et al. 2017). However, Cox et al. (2017) also found that the addition of triclopyr to topramezone did not compromise goosegrass control. In other cool-season species, researchers have evaluated higher rates of both topramezone and triclopyr for smooth crabgrass control and foliar bleaching reduction. They found that the addition of triclopyr reduced the amount of bleached foliage and may have enhanced crabgrass control levels (Brosnan et al. 2014; Yu and McCullough 2016). Chelated iron also has potential as a safener for bermudagrass when applied in combination with topramezone, while ammonium sulfate and turf paint or pigment are ineffective at reducing bermudagrass bleaching (Boyd et al. 2020). Cloquintocet has potential to effectively reduce foliar bleaching in creeping bentgrass turf caused by topramezone, while only chelated iron had positive results at reducing bleaching injury on bermudagrass without increasing or prolonging total injury. Due to inconsistency in the chelated iron results, more evaluations are needed to determine more effective products and programs for use in bermudagrass. More research needs to be conducted in creeping bentgrass to confirm if triclopyr can effectively reduce foliar bleaching caused by topramezone.

Combining HPPD- and PSII-inhibiting herbicides for weed control

There is still a need to find compatible tank-mix partners for topramezone use in bermudagrass turf for goosegrass control. Photosystem II (PSII) inhibiting herbicides have potential as tank-mix partners with HPPD-inhibiting herbicides like topramezone. The combination of the two mode of actions (MOAs) has been shown effective for controlling different weed species as well as resistant weeds in the row crop sector. Weed scientists have

long observed the benefit of combining compatible herbicides to gain an additive or synergistic effect for controlling difficult weeds (Akobundu et al. 1975; Colby 1967; Growing 1960). In the case of combining HPPD- and PSII-inhibitors, the idea is to utilize two herbicides that attack the photosynthetic membranes of plants with both overlapping and differing mechanisms to cause a catastrophic failure of the photosystem which in turn will allow the combos to control weeds more effectively than each herbicide applied alone. Photosystem II-inhibiting herbicides control susceptible weed species by competing for the quinone binding site used by plastoquinone on the D1 protein within the PSII complex (Hess 2000). When the herbicides bind to this site, they effectively stop the electron flow from the PSII complex to the PSI complex, which causes an increase of chlorophyll in the singlet energy state (Hess 2000). This build-up of singlet chlorophyll then proceeds to produce triplet chlorophyll that interacts with oxygen radicals causing singlet oxygen that initiates lipid peroxidation and destruction of chlorophyll and other important organelles (Hess 2000). Typically, both triplet chlorophyll and singlet oxygen are dissipated by carotenoids, but the excessive build-up of singlet and triplet chlorophyll as well as oxygen radicals overloads the carotenoid quenching system leading to severe damage and degradation of carotenoids (Hess 2000). The loss of carotenoids removes the plant's defense to photooxidation. The HPPD-inhibiting herbicides can compound this destruction of the photosynthetic membrane through two mechanisms. First, they reduce or stop the production of plastoquinone allowing for PSII-inhibitors to more efficiently bind to the D1 protein due to less competition (Grossman and Ehrhardt 2007; Hess 2000; Pallett et al. 1998). Second, they stop the production of precursors to carotenoid biosynthesis, which in turn drastically reduces the ability for the plant to replace damaged carotenoids and defend itself from the build-up of excited chlorophyll and oxygen radicals (Grossman and Ehrhardt 2007; Hess 2000; Pallett et al. 1998).

In corn weed management research, mesotrione and atrazine were some of the first HPPD- and PSII-inhibiting herbicides combined and evaluated for synergistic effects on weeds. These admixtures significantly increased control of many weed species including weeds resistant to one or both MOAs (Abendroth et al. 2006; Jhala et al. 2014; Kohrt and Sprague 2017; Woodyard et al. 2009). In multiple studies, mesotrione was applied in combination with different PSII-inhibitors including atrazine, bromoxynil, or metribuzin to evaluate common waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), common lambsquarters (*Chenopodium album L.*), giant ragweed (*Ambrosia trifida L.*), Palmer amaranth (*Amaranthus palmeri S. Watson*), velvetleaf (*Abitulon theophrasti Medik.*), and sunflower (*Helianthus annuus L.*) control. All combinations with mesotrione controlled the broadleaf weeds significantly more than any of the herbicides applied alone (Abendroth et la. 2006). Many researchers have also found that combining mesotrione, tembotrione, or topramezone with atrazine and other PSII-inhibitors can effectively control Palmer amaranth populations resistant to HPPD- or PSII-inhibiting herbicides (Chahal et al. 2019; Jhala et al. 2014; Khort and Sprague 2017).

Researchers have observed similar synergistic combinations in the turfgrass sector as well. Elmore et al. (2013) observed mesotrione plus amicarbazone controlled annual bluegrass (*Poa annua* L.) approximately 70% and greater than each herbicide applied alone. Other studies have shown that admixtures of topramezone and mesotrione with bromoxynil significantly increased Star of Bethlehem (*Ornithogalum umbellatum* L.) control. Since many researchers have observed synergism when utilizing HPPD- and PS2-inhibitors with a variety of weed species, we feel the same is possible when controlling goosegrass with topramezone in bermudagrass. Recently, researchers have observed single applications of topramezone at 12.3 g ai ha⁻¹ plus metribuzin at 420 g ai ha⁻¹ control young to mature goosegrass 75 to 100% and

greater than either topramezone or metribuzin applied alone (Kerr et al. 2019a). Unfortunately, this admixture causes unacceptable bermudagrass injury when topramezone is applied between 12.3 and 24.5 g ai ha⁻¹ (Kerr et al. 2019a; Lindsey et al. 2019). Even though bermudagrass injury was high, the admixture could still be utilized effectively if topramezone rates are lowered significantly in combination with metribuzin. Since topramezone is effective at controlling goosegrass at low rates, it is possible to find an optimum use rate for goosegrass in bermudagrass without causing severe injury (Cox et al. 2017).

Post-treatment Irrigation

One final method that has potential to significantly reduce bermudagrass injury caused by topramezone is post-treatment irrigation. Many past studies have reported that rainfall occurring immediately after foliar-applied herbicides (graminicides, synthetic auxins, photosystem II inhibitors, glutamine synthetase inhibitors, and others) can negatively impact weed control for a wide range of species (Anderson et al. 1993; Behrens and Elakkad 1981; Bovey and Diaz-Colon 1969; Bryson 1988; Doran and Anderson 1975). Even with the negative impacts of immediate rainfall or irrigation, the high sensitivity of goosegrass to low rates of topramezone may allow the use of irrigation to selectively reduce bermudagrass injury to an acceptable level while maintaining effective goosegrass control. Researchers observed that when carfentrazone plus 2,4-D plus dicamba plus MCPP, metribuzin, mesotrione, and simazine were applied with immediate irrigation, the bermudagrass injury was significantly reduced to an acceptable level, but injury caused by topramezone was not reduced by irrigation until four weeks after application (Kerr et al. 2019b). Another study evaluated the influence of immediate posttreatment irrigation on goosegrass control and bermudagrass injury after metribuzin and topramezone applications (Kerr et al. 2019a). The researchers observed that bermudagrass injury

was significantly reduced when treatments were immediately irrigated after metribuzin applied at 480 g ai ha⁻¹, topramezone applied at 12.3 g ai ha⁻¹, or both applied together (Kerr et al. 2019a). The addition of post-treatment irrigation did not reduce goosegrass control when plants were less than three tillers, but once plants became more mature, irrigation decreased control to an unacceptable level (Kerr et al. 2019a). Irrigation can be used effectively to reduce bermudagrass injury caused by topramezone or topramezone plus metribuzin, but more research needs to be completed to determine better irrigation programs that do not compromise goosegrass control.

Research Objectives

- Determine the potential use for topramezone and tembotrione in cool-season and warmseason turfgrass species compared to mesotrione
- 2. Evaluate bermudagrass and goosegrass response to topramezone and metribuzin admixtures, while also determining if differences in absorption, translocation, and metabolism of topramezone occur between bermudagrass and goosegrass
- 3. Evaluate the influence of different post-treatment irrigation timings on bermudagrass and goosegrass response to topramezone plus metribuzin
- 4. Evaluate different low-dose frequent application herbicide programs for goosegrass and smooth crabgrass control on creeping bentgrass greens

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Chapter II. Response of Six Turfgrass Species and Four Weeds to Three HPPD-Inhibiting

Herbicides

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Abbreviations: AUPC, area under the progress curve; HPPD, 4-hydroxyphenylpyruvate dioxygenase; NDVI, normalized difference vegetation index; WAT, weeks after treatment.

Abstract

Mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), tembotrione (2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione), and topramezone ([3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone) are new herbicides that control many troublesome weeds, but little is known about the response of several turfgrass species to these herbicides. A multiyear study was conducted to determine the response of six turfgrass species and four weeds to these three herbicides. Study results generally agreed with previous reports of turfgrass and weed

response to mesotrione, and suggest that tembotrione could be safely used, depending on rate, to control weeds such as smooth crabgrass [Digitaria ischaemum (Schreb.) Schreb. ex Muhl.], broadleaf plantain (*Plantago major* L.), and white clover (*Trifolium repens* L.) selectively in tall fescue [Schedonorus arundinaceus (Schreb.) Dumort., nom. cons.], Kentucky bluegrass (Poa pratensis L.), and zoysiagrass (Zoysia japonica Steud.) turf. Topramezone at 36.8 g a.i. ha⁻¹ controlled smooth crabgrass and white clover better than mesotrione or tembotrione, and smooth crabgrass control by topramezone had similar results as in other studies. Predicted maximum turfgrass injury based on the Gaussian function applied over time generally showed that maximum injury caused by topramezone was less than tembotrione and mesotrione on creeping bentgrass (Agrostis stolonifera L.) and perennial ryegrass (Lolium perenne L.), less than tembotrione and equivalent to mesotrione on tall fescue and perennial ryegrass, equivalent to tembotrione and more than mesotrione on bermudagrass [Cynodon dactylon (L.) Pers. x Cynodon transvaalensis Burtt Davy], and more than tembotrione and mesotrione on zoysiagrass. The area under the progress curve per day of visual injury and normalized difference vegetation index were consistent with trends in predicted maximum injury.

Introduction

Mesotrione, tembotrione, and topramezone inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD) in susceptible species and have become important components of corn (*Zea mays* L.) weed management programs in recent years (Bollman et al., 2008; Mitchell et al., 2001). Mesotrione and topramezone are both labeled for use in several turfgrass species to control or suppress more than 40 weeds (Syngenta Crop Protection, 2011; BASF Corporation, 2014; Bollman et al., 2008; Soltani et al., 2012) Unlike topramezone and mesotrione, tembotrione is only registered in corn. It is labeled to control or suppress more than 60 weeds common to cropping systems, and several of those weeds are also found in turfgrass systems (Bayer CropScience, 2012; Santel, 2009).

Although mesotrione and topramezone are labeled for use in turfgrass, only mesotrione has had thorough research published that supports its use in multiple turf species (Beam et al., 2006; Brosnan et al., 2010; Goddard et al., 2010; Jones and Christians, 2007; McElroy et al., 2007; McElroy and Walker, 2009; Post et al., 2013; Williams et al., 2009; Willis et al., 2006, 2007). Field research involving mature turfgrass response to postemergence applications of topramezone has only been reported for Kentucky bluegrass and tall fescue (Brosnan et al., 2010, 2013; Brosnan and Breeden, 2013; Elmore et al., 2012). Seedlings of creeping bentgrass, perennial ryegrass, and tall fescue were also not injured by topramezone applied at 0, 2, 4, and 6 wk after seeding (Johnston et al., 2016). In addition, topramezone effects on small plugs of creeping bentgrass and bermudagrass have been evaluated in greenhouse studies (Brosnan et al., 2011; Elmore et al., 2011a, 2011b, 2015). Only one field study has been published with tembotrione comparing dallisgrass (*Paspalum dilatatum* Poir.) control and tall fescue tolerance, and a few greenhouse studies have evaluated the response of small bermudagrass plugs to

tembotrione and other HPPD inhibitors (Brosnan et al., 2011; Elmore et al., 2011a, 2011b, 2013).

Turf systems often include numerous turfgrass species managed as mixtures or abutting monocultures of divergent species that may vary in herbicide susceptibility. Even though topramezone is already registered for use in some turfgrasses, there is need for research to evaluate the response of several turfgrass species to topramezone and to evaluate the potential of tembotrione for registration in turfgrass. Therefore, experiments were conducted to compare different rates of topramezone and tembotrione, each applied once or twice, with mesotrione at a standard rate applied once or twice for broadleaf plantain, buckhorn plantain (*Plantago lanceolata* L.), smooth crabgrass, and white clover control and turfgrass response in mature stands of bermudagrass, creeping bentgrass, Kentucky bluegrass, perennial ryegrass, tall fescue, and zoysiagrass.

Materials and Methods

Six field experiments were conducted in Blacksburg, VA, in 2007 and 2014 to evaluate the response of six turfgrass species and weeds to three HPPD inhibitors. Specific cultivars and locations for each of the six turfgrass species in each year are listed in Table 1. Tall fescue was mown weekly at 8.9 cm, and all other turfgrass species were mown three times per week at 1.5 cm. All study sites at the Virginia Tech Turfgrass Research Center and Glade Road Research Facility for both years followed an integrated program using irrigation, fertility, and pest control to promote appropriate turf health. Soil at the Turfgrass Research Center was a Groseclose—Urban land complex loam (clayey, mixed, mesic Typic Hapludult) with a pH of 6.2 and 4.1% organic matter. The soil at the Glade Road Research Facility was a Duffield silt loam (a fine-loamy, mixed, active, mesic, Ultic Hapludalf)—Ernest silt loam (a fine-loamy, mixed,

superactive, mesic Aquic Fragiudult) complex, with a pH of 6.6 and 3.9% organic matter. All experiments were arranged as randomized complete block designs with three replications. Plots were 1.8 by 1.8 m in 2007 and 0.9 by 1.8 m in 2014. Treatments were arranged as a two by seven factorial, with two levels of application frequency (one and two applications) and the seven levels of the herbicide factor including topramezone and tembotrione each at three rates and compared with mesotrione at a standard rate. Herbicide levels included topramezone (Pylex herbicide, BASF Corporation) at 18.4, 36.8, and 55.2 g a.i. ha⁻¹, tembotrione (Laudis herbicide, Bayer CropScience) at 138, 276, and 414 g a.i. ha⁻¹, and mesotrione (Tenacity herbicide, Syngenta Crop Protection) at 280 g a.i. ha⁻¹. The mesotrione rate and the middle rate of topramezone represent the maximum labeled rates for turfgrass (Syngenta Crop Protection, 2011; BASF Corporation, 2014). The lowest and middle rates of tembotrione represent the standard and maximum labeled rates for field corn (Bayer CropScience, 2012). An untreated check was included for comparison. Herbicide application times are listed in Table 1. All treatments were mixed in pH 7 water with 0.25% nonionic surfactant (Induce surfactant, Helena Chemical Co.) by volume and applied using a CO₂-pressurized sprayer calibrated to deliver 280 L ha⁻¹ at 289 kPa via Teejet flat fan 11004 nozzles (Teejet Technologies).

Treatment effects on all six turfgrass species in both years were evaluated weekly for 6 wk. Turfgrass injury was assessed visually on a scale from 0 to 100%, where 0 equals no injury and 100 equals complete plant necrosis. Turf quality was assessed at all evaluation dates and based on a 1 to 9 scale, where 9 is ideal turf quality, 6 is minimally acceptable turf quality, and 1 is a complete loss of green turf. Turf spectral reflectance at 650 nm (red) and 880 nm (near infrared) were recorded by a Crop Circle ACS-210 from a height 44 cm above the canopy level (Holland

Scientific). The normalized difference vegetative index (NDVI) was calculated as a measure of overall canopy cover.

Weed control was also assessed in the 2007 and 2014 studies. Weeds evaluated included broadleaf plantain, buckhorn plantain, smooth crabgrass, and white clover. Weed growth stages and cover percentage when treated, year, and type of turfgrass where the weed was evaluated are listed in Table 2. Weed control was assessed visually on a scale of 0 to 100, where 0 is no control and 100 is complete plant necrosis, at weekly intervals as described for other response variables.

Turfgrass injury and NDVI measurements with time were converted to area under the progress curve (AUPC) using

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

where ∂ is the AUPC, i is the ordered sampling date, ni is the number of sampling dates, y is turf injury or NDVI measurement at a given date, and t is the time in days. The AUPC was then converted to the average per day. Campbell and Madden (1990) applied this equation to disease epidemiology, and Askew et al. (2013) utilized it for weediness with time in a turfgrass comparison study. The AUPC is useful in situations where turfgrass response for long durations is assessed by repeated measures. This technique offers a better comparison between treatments when both severity and duration of the measured response is important compared with averages with time and single-date analysis.

Maximum turfgrass injury was predicted using the Gaussian function applied to turf injury percentage with time on each experimental unit:

$$y = ae^{(\frac{-(x-b)^2}{2c^2})},$$
 [2]

where a is the maximum turfgrass injury in a given experimental unit, b is the number of days after treatment at which maximum injury occurred, and c controls the width of the curve. The fit of the curve was based on least sums of squares using the Gauss–Newton iterative method of PROC NLIN, with estimated parameters a and b constrained to be positive values not greater than 100% and 42 d, respectively.

Data for each response variable were tested for normality using PROC UNIVARIATE and the Shapiro-Wilk statistic in SAS 9.2 (SAS Institute, 2009) and homogeneity of variance was confirmed by visually inspecting plotted residuals and other metrics using the DIAGNOSTIC option in PROC PLOT of SAS 9.2. Homogeneity of variance was further assessed using Levene's test, where one-way ANOVAs for main effects or all possible combinations of factorial levels were tested using the HOVTEST WELCH option in the MEANS statement of PROC GLM in SAS 9.2. When needed based on significance (P < 0.05) in Levene's test, data were transformed to the log or arcsin square root to meet assumptions of ANOVA. In such cases where transformation was needed, data were back transformed for presentation clarity. A combined ANOVA was conducted separately for each turfgrass species, with sums of squares partitioned to evaluate block, year, application frequency, herbicide treatment, application frequency by herbicide treatment, and the interaction of year with all other fixed effects and interactions. Year was considered random, and mean square of treatment effects were tested using mean square associated with each effect interaction with the random variable (McIntosh, 1983). Appropriate means were separated using Fisher's protected LSD at $\alpha = 0.05$.

Results and Discussion

Turfgrass Injury Response with Time

The interaction of year \times herbicide was significant (P < 0.0001) for average injury AUPC of all six turfgrass species except creeping bentgrass (P = 0.0689) and zoysiagrass (P = 0.0922); therefore, data were pooled across years to compare the significant herbicide main effect for creeping bentgrass (P = 0.0002) and zoysiagrass (P = 0.0006). Although the frequency of application did not interact with herbicide treatment ($P \ge 0.1211$), the effect of application frequency was significant for injury AUPC per day for all species and dependent on year for all but perennial ryegrass. Thus, the main effect of application frequency is reported for perennial ryegrass (P = 0.0425) and the interaction of year × application frequency is reported for all other species ($P \le 0.0385$). Kentucky bluegrass and tall fescue were among the more tolerant turfgrass species to the herbicides tested. Kentucky bluegrass injury AUPC was not more than 5% d⁻¹ for any herbicide treatment except the high rate of tembotrione, which had an injury AUPC of 21% d⁻¹ in 1 of 2 yr (Table 3). Tembotrione was also injurious to tall fescue when applied at the middle and high rates primarily in 2014 (24 and 37% injury d⁻¹, respectively), while in 2007 the two rates injured tall fescue <13% (Table 3). The reasons for these differences are unclear, but possible sources of error between years include different tall fescue cultivars, slightly more rainfall in 2014, and differences in turfgrass age (6 yr in 2014 vs. 4 yr in 2007). When comparing mesotrione and the middle rates of tembotrione and topramezone, all representing maximum labeled rates for each herbicide, topramezone had the highest injury AUPC per day for bermudagrass and zoysiagrass, while tembotrione had the highest injury AUPC per day for perennial ryegrass (Table 3). Mesotrione and tembotrione injured creeping bentgrass similarly, but greater than topramezone. When averaged across herbicide treatments, injury AUPC per day increased with increasing application frequency for all six turfgrass species regardless of year (Table 3).

Topramezone and mesotrione did not injure tall fescue more than 15% in other field studies (Brosnan and Breeden, 2013; Brosnan et al., 2010; Elmore et al., 2012), which is similar to the observed injury from mesotrione and the middle rate of topramezone in the current study (data not shown). This similarity is evident by the relatively low injury AUPC per day of not greater than 13% (Table 3). Elmore et al. (2013) observed no significant tall fescue injury from mesotrione, tembotrione, or topramezone at 280, 92, and 37 g a.i. ha⁻¹, respectively, following 9 of 10 applications over 2 yr. Although the tembotrione rate used by Elmore et al. (2013) is less than half of the middle rate in this study, the mesotrione and topramezone rates are similar, and the lack of tall fescue injury from these herbicides is in agreement with the data from our present study (Table 3). When topramezone and tembotrione were applied at 38 and 276 g ha⁻¹, respectively, bermudagrass plugs maintained in a greenhouse exhibited average AUPC of 35 to 39% d⁻¹ based on applying the AUPC equation to means of observed visual bleaching reported by Brosnan et al. (2011) and Elmore et al. (2011a, 2011b). In the current field trials, topramezone and tembotrione at the same rates caused AUPC of 47 and 37% d⁻¹ injury, respectively (Table 3). Although there is no suitable comparison for long-term injury response on other turfgrass species, topramezone at 37 g ha⁻¹ injured creeping bentgrass 13 and 27% 2 wk after treatment (WAT) in two greenhouse trials (Elmore et al., 2015). The data from this study generally suggest that tembotrione would be safe for use in tall fescue, Kentucky bluegrass, and zoysiagrass but may be too injurious to creeping bentgrass, bermudagrass, and perennial ryegrass (Table 3). The data from the present studies concur with the results of other researchers regarding tall fescue (Brosnan and Breeden, 2013; Elmore et al., 2012) and bermudagrass response (Brosnan et al., 2011; Elmore et al., 2011a, 2011b) to topramezone and tembotrione.

Maximum Turfgrass Injury

Maximum turfgrass injury, as predicted during the 42-d assessment period by the Gaussian function, exhibited a significant herbicide \times application frequency interaction (P < 0.0001) for all species (Table 4). Year interactions were not significant ($P \ge 0.0987$), so the herbicide \times application frequency interaction is shown in Table 4 pooled across years. While injury AUPC per day accounts for cumulative injury effects with time, predicted maximum injury gives an appreciation for the severity of injury caused by some treatments. The two responses were in general agreement though, because many of the trends in response between herbicide treatments within a given species are similar (Tables 3 and 4). For example, tembotrione was generally more injurious to creeping bentgrass, Kentucky bluegrass, perennial ryegrass, and tall fescue than topramezone, and topramezone was more injurious to zoysiagrass than tembotrione (Table 4). For creeping bentgrass, bermudagrass, and zoysiagrass, predicted maximum injury increased with increasing application frequency for all herbicide treatments (Table 4). Generally, more injurious treatments as noted with injury AUPC per day data (Table 3) tended to have significant differences in predicted maximum injury between application frequencies because two applications tended to cause stand reduction and more severe injury. Predicted maximum injury from the middle rate of topramezone was equivalent to mesotrione for tall fescue and Kentucky bluegrass, lower than mesotrione for creeping bentgrass and perennial ryegrass, and greater than mesotrione for bermudagrass and zoysiagrass (Table 4).

Similar field trials reported mesotrione injuring creeping bentgrass >96% after two applications (Beam et al., 2006; Branham et al., 2005; Jones and Christians, 2007). In the current study, mesotrione injured creeping bentgrass a predicted maximum of 98% after two applications compared with 89% from a single application (Table 4). Topramezone at 36.8 g ha⁻¹ applied as a

single application injured creeping bentgrass at a predicted maximum of 70% (Table 4), while Elmore et al. (2015) observed topramezone at the same rate only injuring creeping bentgrass approximately 26% in the greenhouse. Elmore et al. (2015) assessed injury at 2 wk after treatment, which is similar to the timing of predicted maximum injury by topramezone at 37 g a.i. ha^{-1} in this study. The timing was 12 d after treatment based on the estimated parameter b in the Gaussian equation when regressed over the 42-d duration of the study (data not shown). The researchers also found mesotrione, tembotrione, and topramezone applied in a single application injured bermudagrass a maximum of 22, 58, and 58%, respectively, which is lower than the maximum turfgrass injury observed in the present study (Brosnan et al., 2011; Elmore et al., 2011a, 2011b). In the field studies at Virginia Tech, the maximum predicted injury caused to bermudagrass by mesotrione, tembotrione, and topramezone at one application was 66, 71, and 81%, respectively (Table 4), which agrees with the observed injury values (data not shown). In three separate field studies, topramezone and mesotrione injured tall fescue <15% (Brosnan et al., 2010; Brosnan and Breeden, 2013; Elmore et al., 2012), while Elmore et al. (2013) reported that mesotrione, tembotrione, and topramezone caused maximum injury of 5, 18, and 33%, respectively, at 4 WAT. Topramezone at 37 g ha⁻¹ injured tall fescue at a predicted maximum of 20% (Table 4), which is similar to the 15% injury reported from the Knoxville, TN, field studies (Brosnan and Breeden, 2013; Brosnan et al., 2010; Elmore et al., 2012). Although we observed injury levels with tembotrione on tall fescue similar to those of Elmore et al. (2013), we cannot make a direct comparison of tall fescue injury between the studies because our closest comparable rate was two times higher than theirs. Mesotrione at 280 g a.i. ha⁻¹ did not injure perennial ryegrass and Kentucky bluegrass more than 18% in other field trials (Beam et al., 2006; Branham et al., 2005; Elmore et al., 2013; Golob et al., 2013; McCurdy et al., 2008; Willis et al., 2007), and the data from the current study show mesotrione causing similar predicted maximum injury to perennial ryegrass and Kentucky bluegrass (Table 4). There have been no studies published evaluating mesotrione, tembotrione, or topramezone in zoysiagrass to which we can compare the present results.

Turf Normalized Difference Vegetation Index

The herbicide main effect on NDVI AUPC per day was significant for bermudagrass, creeping bentgrass, Kentucky bluegrass, tall fescue, and zoysiagrass ($P \le 0.0290$), while the interaction of herbicide \times application frequency was significant for perennial ryegrass (P =0.0035). The main effect of application frequency on NDVI AUPC per day was also significant $(P \le 0.0112)$ for all six turfgrass species. The NDVI is used to evaluate turfgrass quality by measuring the difference in spectral reflectance between red and near-infrared wavelengths (Bell et al., 2002; Bremer et al., 2011), so the higher the NDVI reading, the higher the turf quality or health and vice versa. Trends between herbicides for NDVI AUPC per day were inversely related to both injury AUPC per day and predicted maximum injury (Tables 3, 4, and 5). For example, NDVI AUPC per day from the middle rate of tembotrione was lower than that of topramezone for creeping bentgrass, perennial ryegrass, and tall fescue; higher than topramezone for zoysiagrass; and equivalent to topramezone for bermudagrass and Kentucky bluegrass (Table 5). These trends are the same as predicted maximum injury for all turfgrass species (Table 4). In many cases, such as with bermudagrass, creeping bentgrass, tall fescue, and zoysiagrass, NDVI AUPC per day was inversely related to both tembotrione and topramezone rates (Table 5). Brosnan et al. (2011) reported that chlorophyll a and b of treated bermudagrass plugs in the greenhouse were reduced equivalently by tembotrione and topramezone but more than by mesotrione, which is a similar trend to NDVI AUPC per day in the present studies (Table 5). The NDVI has been correlated with chlorophyll content in cropping and turfgrass systems (Gitelson et al., 2003; Stiegler et al., 2005).

Weed Control

The interaction of herbicide treatment × application frequency was significant for broadleaf plantain (P = 0.0024) and smooth crabgrass (P = 0.0178); the interaction of year × herbicide treatment was significant for broadleaf plantain (P < 0.0001); and the main effect of herbicide treatment was significant for white clover (P = 0.0110). The main effect of application frequency was also significant for broadleaf plantain (P = 0.0376), smooth crabgrass (P = 0.0418), and white clover (P = 0.0015) control; and the interaction of year × application frequency was significant for buckhorn plantain (P < 0.0001) control (Table 6).

When herbicides were applied twice rather than once, broadleaf plantain and smooth crabgrass control increased for all herbicide treatments, and the control of all weeds increased when averaged across herbicide treatment, regardless of year (Table 6). When applied once, topramezone at 36.8 g a.i. ha⁻¹ controlled smooth crabgrass and white clover more than mesotrione at 280 g a.i. ha⁻¹ and tembotrione at 276 g a.i. ha⁻¹ (Table 6). Buckhorn plantain control varied between years, with generally less control in 2007 (Table 6). In 2014 when averaged across application frequency, buckhorn plantain was not controlled more than 58% by any herbicide, while the average buckhorn plantain control from all herbicides applied twice in 2014 was 64% (Table 6). Broadleaf plantain was not controlled by single applications of any herbicide treatment (Table 6). When applied twice, tembotrione controlled broadleaf plantain 81 to 98% depending on rate, which was typically greater than mesotrione or topramezone (Table 6). White clover control was 1.5 times greater when herbicides were applied twice vs. when applied once (Table 6). When averaged across application frequencies, white clover control by

tembotrione exhibited a positive response to increasing rate, with the middle rate being equivalent to mesotrione and less than all rates of topramezone (Table 6).

There are no reports in the scientific literature of turfgrass studies evaluating tembotrione for controlling any of these weed species or for topramezone controlling broadleaf plantain, buckhorn plantain, or white clover, but mesotrione has been reported to control broadleaf plantain and white clover seedlings. Mesotrione has also been compared with topramezone for mature smooth crabgrass control. The average smooth crabgrass control by mesotrione at 280 g ha⁻¹ and topramezone at 9 g ha⁻¹ in fallow field sites during 2 yr was 47 to 69% depending on the N fertilizer rate at 6 WAT in a study by Elmore et al. (2012). In the studies at Virginia Tech, an average of 65% smooth crabgrass control was based on six sites that comprised creeping bentgrass, Kentucky bluegrass, and perennial ryegrass turf maintained at 1.5 cm for 2 yr each, which was within the range of smooth crabgrass control reported by Elmore et al. (2012). The lowest rate of topramezone in the current studies was half the label-recommended rate (BASF Corporation, 2014), which was twice that of previous research (Elmore et al., 2012), and controlled smooth crabgrass 78% 6 WAT (Table 6). In mature Kentucky bluegrass, perennial ryegrass, and tall fescue turf maintained at 6.35 cm, mesotrione at 140 g a.i. ha⁻¹ controlled smooth crabgrass 55 to 73% at 5 WAT depending on location (Post et al., 2013), which control range spans that observed for mesotrione at 280 g ha⁻¹ in the current study (Table 6). Studies conducted in Knoxville, TN, and West Lafayette, IN, demonstrated that topramezone at 24 g ha⁻¹ controlled smooth crabgrass inconsistently 9 WAT, where 77 and 17% control was noted at each respective location (Brosnan et al., 2013). In this study at 6 WAT, topramezone at 18.4 g ha⁻¹ controlled smooth crabgrass (Table 6) similar to the Knoxville, TN, trial site. Willis et al. (2007) reported that one application of mesotrione at 280 g ha⁻¹ applied under field and greenhouse

conditions controlled white clover 46% 6 wk after a single application, which is similar to the 65% white clover control observed by the same mesotrione rate and assessment timing in the current study (Table 6).

The data from the Blacksburg studies are consistent with earlier reports related to mesotrione safety in Kentucky bluegrass, perennial ryegrass, and tall fescue (Brosnan et al., 2010; Brosnan and Breeden, 2013; Elmore et al., 2012; Post et al., 2013), injury to bermudagrass and creeping bentgrass (Beam et al., 2006; Brosnan et al., 2011; Elmore et al., 2011a, 2011b), and weed control efficacy on smooth crabgrass and white clover (Brosnan et al., 2013; Elmore et al., 2012; Post et al., 2012). The present study further shows that mesotrione may be safely applied once to zoysiagrass (Tables 3 and 4), but it does not effectively control broadleaf plantain and buckhorn plantain (Table 6). The results also suggest that tembotrione could be safely used, depending on rate, to control weeds such as broadleaf plantain, smooth crabgrass, and white clover selectively in Kentucky bluegrass, tall fescue, and zoysiagrass (Tables 3, 4, and 6). These findings coincide with reports of tall fescue safety at low rates of tembotrione (Elmore et al., 2013). The results also suggest that topramezone at 36.8 g a.i. ha⁻¹ controls smooth crabgrass and white clover better than mesotrione or tembotrione and generally agree with smooth crabgrass control data observed from topramezone in other studies (Brosnan et al., 2013). The results from these studies follow similar reports that topramezone is generally safe for use in tall fescue (Brosnan et al., 2013; Elmore et al., 2013) and less injurious to creeping bentgrass than mesotrione (Beam et al., 2006; Elmore et al., 2015), although the maximum predicted injury to creeping bentgrass by topramezone exceeds that reported by Elmore et al. (2015). The results further show that topramezone is safer than mesotrione and tembotrione on perennial ryegrass and safe for use in Kentucky bluegrass (Tables 3 and 4). We have also shown that topramezone is more injurious to

bermudagrass and zoysiagrass than mesotrione (Tables 3 and 4). These studies will help turfgrass managers assess herbicidal risks near areas that contain potentially sensitive turfgrass species, improve our understanding of weed control efficacy in managed turf from HPPD-inhibiting herbicides, and support considerations for label expansion or registration of the tested herbicides.

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TablesTable 1. Turfgrass species, cultivars, locations, and application dates. Tall fescue was mown weekly at 8.9 cm and all other turfgrass species were mown three times per week at 1.5 cm.

Species	Cultivar	Establishment year	Location†	Initial application	Sequential application
Bermudagrass	Vamont	1995	TRC	17 Aug. 2007	7 Sept. 2007
	Patriot	2005	GRF	9 July 2014	30 July 2014
Creeping bentgrass	L93	2002	TRC	31 Aug. 2007	21 Sept. 2007
	L93	2004	GRF	8 Aug. 2014	29 Aug. 2014
Kentucky bluegrass	Midnight	2004	GRF	25 Aug. 2007	15 Sept. 2007
	Midnight II	2008	GRF	9 July 2014	30 July 2014
Perennial ryegrass	Prosport	2004	GRF	25 Aug. 2007	15 Sept. 2007
	blend‡	2009	GRF	9 July 2014	30 July 2014
Tall fescue	Falcon III	2003	GRF	5 Sept. 2007	26 Sept. 2007
	Falcon IV	2008	GRF	9 July 2014	30 July 2014
Zoysiagrass	Meyer	2001	TRC	17 Aug. 2007	7 Sept. 2007
	Meyer	2008	GRF	8 Aug. 2014	29 Aug. 2014

[†] GRF, Glade Road Research Facility; TRC, Turfgrass Research Center.

[‡] Perennial ryegrass blend included 39% 'Federation,' 30% 'Partner,' and 29% 'Cadence.'

Table 2. Weed species, trial year, turfgrass species, median weed growth stage, and weed cover percentage at trial initiation.

Weed species	Year†	Turfgrass species	Median weed stage	Weed cover
				%
Broadleaf plantain	2007	tall fescue	mature, senesced bloom	$3.1 \pm 0.5 \ddagger$
	2014	tall fescue	mature, blooming	20 ± 1.6
Buckhorn plantain	2007	zoysiagrass	mature, senesced bloom	7.8 ± 0.8
	2014	zoysiagrass	mature, senesced bloom	9.1 ± 1.4
Smooth crabgrass	2007	creeping bentgrass	12-leaf stage, prebloom	6.6 ± 0.56
	2014	creeping bentgrass	30-tiller stage + seed heads	18 ± 0.94
	2007	perennial ryegrass	28-tiller stage + seed heads	44 ± 2.5
	2014	perennial ryegrass	25-tiller stage + seed heads	51 ± 2.2
	2007	Kentucky bluegrass	30-tiller stage + seed heads	59 ± 2.5
	2014	Kentucky bluegrass	15-tiller stage + seed heads	22 ± 2.5
White clover	2007	tall fescue	mature, senesced bloom	49 ± 1.2
	2014	tall fescue	mature, blooming	33 ± 1.9
	2007	Kentucky bluegrass	mature, senesced bloom	18 ± 1.8
	2014	Kentucky bluegrass	mature, blooming	37 ± 2.3

[†] See Table 1 for trial initiation dates.

 $[\]ddagger$ Mean \pm standard error.

Table 3. Daily average during a 42-d period of the area under the progress curve (AUPC) based on visually estimated turfgrass injury from seven herbicide treatments and two application frequencies shown as the main effect of herbicide treatment for creeping bentgrass (AGSST) and zoysiagrass (ZOYJA); interaction of year × herbicide treatment for bermudagrass (CYNDA), tall fescue (FESAR), perennial ryegrass (LOLPE), and Kentucky bluegrass (POAPR); main effect of application frequency for LOLPE; and interaction of year × application frequency for AGSST, CYNDA, FESAR, POAPR, and ZOYJA. Turfgrass species were managed as field turf and studies conducted at unique locations in 2007 and 2014 in Blacksburg, VA. Turfgrass cultivars, initial application dates, and specific locations are shown in Table 1. Tall fescue was mown weekly at 8.9 cm and all other turfgrass species were mown three times per wk at 1.5 cm.

		AUPC†											
				CYI	NDA	FES	SAR LOLPE		POAPR				
Herbicide‡	Rate	AG	SST	2007	2014	2007	2014	2007	2014	2007	2014	ZO	YJA
	g a.i. ha ⁻¹			_			%	d^{-1} ——					
Mesotrione	280	6	4	42	35	6	13	5	21	0.2	4	1	3
Tembotrione	138	5	5	24	54	4	8	6	19	0.2	2	1	2
	276	6	6	37	60	11	24	13	43	0.3	5	1	9
	414	6	9	44	61	12	37	15	47	0.8	21	2	7
Topramezone	18.4	1	8	41	60	4	5	1	2	0.1	0	3	0
	36.8	2	4	47	66	10	6	1	17	0.1	2	4	5
	55.2	3	0	52	70	13	14	5	24	0.1	2	4	8
LSD (0.05)	_	4	4	5	4	4	8	2	9	0.5	8	3	3
Frequency		2007	2014					A	vg.			2007	2014
One application	_	23	53	27	46	7	8	9)	0.1	2	12	25
Two applications	_	44	66	55	70	10	23	2	0.	0.4	8	34	42
LSD (0.05)	_	3	3	3	2	2	5	2	2	0.2	4	2	3

[†] Turfgrass injury was based on a visually estimated percentage reduction of apparent healthy tissue relative to the untreated check, where 0% is healthy tissue equivalent to the untreated check and 100% is complete death of all treated tissue, and assessed weekly for 6 wk. Data were converted to the AUPC for the 42-d assessment period and then converted to the daily average of AUPC.

[‡] Herbicides applied with 0.25% (v/v) nonionic surfactant at 280 L ha⁻¹ and 289 kPa once or twice at a 3-wk interval. Means for herbicide treatments are averaged across two application frequencies, and means for application frequencies are averaged across seven herbicide treatments. Differences between means within a column for either

herbicide treatment or application frequency that are greater than or equal to the LSD indicate that the means are significantly different based on Fisher's protected LSD test at P < 0.05.

Table 4. Predicted maximum turfgrass injury during a 42-d period using the Gaussian function applied to visually estimated turfgrass injury shown as the interaction of seven herbicide treatments and two application frequencies (F1, only one application; F2, two applications at a 3-wk interval) for creeping bentgrass (AGSST), bermudagrass (CYNDA), tall fescue (FESAR), perennial ryegrass (LOLPE), Kentucky bluegrass (POAPR), and zoysiagrass (ZOYJA).

Turfgrass species were managed as field turf and studies conducted at unique locations in 2007 and 2014 in Blacksburg, VA. Turfgrass cultivars, initial application dates, and specific locations are shown in Table 1. Tall fescue was mown weekly at 8.9 cm and all other turfgrass species were mown three times per wk at 1.5 cm.

		Predicted maximum turfgrass injury†											
		AGSST		CYNDA		FESAR		LOLPE		POAPR		ZOYJA	
Herbicide‡	Rate	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
	g a.i. ha ⁻¹							- %					
Mesotrione	280	89	98*	66	83*	19	23	22	35*	3	0	8	36*
Tembotrione	138	83	97*	64	81*	12	15	21	50*	1	12*	13	31*
	276	88	100*	71	89*	21	33*	37	76*	0	17*	16	60*
	414	90	100*	82	93*	28	49*	52	82*	22	28*	36	67*
Topramezone	18.4	59	56	75	87*	13	12	4	1	0	0	52	75*
•	36.8	70	76*	81	93*	20	19	13	14	2	4	62	93*
	55.2	75	81*	88	93*	25	32*	14	36*	5	6	77	96*
LSD (0.05)	_	4	2	2	2	7	7	5	6	6	4	4	3

^{*} The effect of application frequency within a given herbicide treatment for turfgrass injury is statistically significant at P < 0.05.

[†] Turfgrass injury was based on a visually estimated percentage reduction of apparent healthy tissue relative to the untreated check, where 0% is healthy tissue equivalent to the nontreated check and 100% is complete death of all treated tissue, and assessed weekly for 6 wk. Data from all rating dates were converted to predicted maximum percentage turfgrass injury using parameter a from the Gaussian function, $y = a \exp[-(x - b)^2/2c^2]$, where y is predicted percentage injury, x is d after treatment, a is maximum injury, b is the day at which maximum injury occurred, and c controls the width of the curve. Fit of the curve was based on least sums of squares with a and b constrained to positive values not greater than 100% and 42 d, respectively.

‡ Herbicides applied with 0.25% (v/v) nonionic surfactant at 280 L ha⁻¹ and 289 kPa. Differences between means of herbicide treatments within a column that are greater than or equal to the LSD indicate that the means are significantly different based on Fisher's protected LSD test at $P \le 0.05$.

Table 5. Daily average during a 42-d period of the area under the progress curve (AUPC) based on the normalized difference vegetation index (NDVI) from seven herbicide treatments and two application frequencies shown as the main effect of herbicide treatment for creeping bentgrass (AGSST), bermudagrass (CYNDA), tall fescue (FESAR), Kentucky bluegrass (POAPR), and zoysiagrass (ZOYJA); the interaction of herbicide treatment × application frequency for perennial ryegrass (LOLPE); and the main effect of application frequency for all species.

Turfgrass species were managed as field turf and studies conducted at unique locations in 2007 and 2014 in Blacksburg, VA. Turfgrass varieties, initial application dates, and specific locations are shown in Table 1. Tall fescue was mown weekly at 8.9 cm and all other turfgrass species were mown three times per wk at 1.5 cm.

					AUPC†	_PE§								
Herbicide‡	Rate	AGSST	CYNDA	FESAR	<u>F1</u>	F2	POAPR	ZOYJA						
	g a.i. ha ⁻¹				% d ⁻¹ -									
Mesotrione	280	0.525	0.515	0.661	0.635	0.618	0.642	0.570						
Tembotrione	138	0.551	0.490	0.673	0.694	0.6389	0.674	0.618						
	276	0.542	0.471	0.637	0.609	0.531	0.640	0.577						
	414	0.495	0.469	0.610	0.611	0.471	0.568	0.570						
Topramezone	18.4	0.701	0.483	0.655	0.725	0.734	0.705	0.562						
•	36.8	0.655	0.479	0.672	0.662	0.644	0.645	0.553						
	55.2	0.632	0.465	0.660	0.623	0.631	0.649	0.540						
LSD (0.05)	_	0.0538	0.0120	0.0384	0.0618	0.0526	0.0465	0.0214						
Frequency‡					A	vg.								
One application	_	0.608	0.496	0.669	0.6		0.673	0.577						
Two applications	_	0.563	0.467	0.636	0.610		0.619	0.561						
LSD (0.05)	_	0.0288	0.00640	0.0205	0.0200		0.0200		0.0200		0.0200		0.0249	0.0114

[†] Turf NDVI was assessed weekly for 6 wk using a multispectral analyzer (Crop Circle Model ACS-210, Holland Scientific). Data were converted to the AUPC for the 42-d assessment period and then converted to the daily average of AUPC. The average NDVI AUPC d^{-1} of untreated turf \pm SE was 0.7538 ± 0.0030 , 0.5995 ± 0.0114 , 0.715 ± 0.0071 , 0.7992 ± 0.0021 , 0.7536 ± 0.0041 , and 0.6647 ± 0.0103 for AGSST, CYNDA, FESAR, LOLPE, POAPR, and ZOYJA, respectively.

[‡] Herbicides applied with 0.25% (v/v) nonionic surfactant at 280 L ha⁻¹ and 289 kPa. Means for herbicide treatment main effects are averaged across two application frequencies, and means for application frequency main effects are

averaged across seven herbicide treatments. All data are averaged across 2 yr. Differences between means within a column for either herbicide treatment or application frequency that are greater than or equal to the LSD indicate that the means are significantly different based on Fisher's protected LSD test at $P \le 0.05$.

§ F1, frequency of only one application; F2, frequency of two applications applied at a 3-wk interval.

Table 6. Visually estimated percentage weed control§ 6 wk after initial treatment shown as the interaction of herbicide treatment × application frequency for smooth crabgrass (DIGIS) and broadleaf plantain (PLAMA), the interaction of year × herbicide treatment for buckhorn plantain (PLALA), the main effect of herbicide treatment for white clover (TRFRE), the main effect of application frequency for DIGIS and PLAMA, and the interaction of year × application frequency for PLALA and TRFRE.

		Weed control†								
	_	DIGIS§		PLALA		PLAMA§				
Herbicide‡	Rate	F1	F2	2007	2014	F1	F2	TRFRE		
	g a.i. ha ⁻¹			%	ó ———					
Mesotrione	280	65	71*	10	50	3	59*	62		
Tembotrione	138	58	84*	8	58	0	81*	52		
	276	61	85*	15	53	3	94*	61		
	414	65	83*	21	57	4	98*	79		
Topramezone	18.4	78	92*	8	15	0	47*	81		
-	36.8	87	93*	9	16	0	68*	90		
	55.2	94	96*	6	23	0	77*	92		
LSD (0.05)	_	14	21	14	20	NS	20	12		
Frequency		A	vg.			Av	vg.	Avg.		
One application	_		95	4	13	2		60		
Two applications	_	1	00	18	64	7	1	93		
LSD (0.05)	_		3	7	11	4	5	8		

^{*}The effect of application frequency within a given herbicide treatment for weed control is statistically significant at P < 0.05. NS, not significant.

† Weed control was based on a visually estimated percentage reduction of apparent healthy weed tissue relative to the untreated check, where 0% is healthy tissue equivalent to the untreated check and 100% is complete necrosis of all treated weed tissue. Means for DIGIS control are pooled across six unique locations that consisted of perennial ryegrass, Kentucky bluegrass, and creeping bentgrass turf in 2 yr each; PLALA control was assessed in zoysiagrass turf each year; PLAMA control was assessed in tall fescue turf and is pooled across 2 yr; means for TRFRE control are pooled across four unique locations that consisted of Kentucky bluegrass and tall fescue turf in 2 yr each.

‡ Herbicides applied with 0.25% (v/v) nonionic surfactant at 280 L ha⁻¹ and 289 kPa. Differences between means of herbicide treatments or application frequencies within a column that are greater than or equal to the LSD indicate that the means are significantly different based on Fisher's protected LSD test at $P \le 0.05$.

§ F1, frequency of only one application; F2, frequency of two applications applied at a 3-wk interval.

Short title: Low-rate topramezone admixture

Chapter III. Differences in selectivity between bermudagrass and goosegrass (*Eleusine indica*) to low-rate topramezone and metribuzin combinations

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Abstract

Goosegrass [*Eleusine indica* (L.) Gaertn.] remains problematic for bermudagrass [*Cynodon dactylon* (L.) Pers.] turf managers due to available postemergence herbicides being ineffectual for selective control of mature plants, and lack of sufficient residual activity from those herbicides that control seedling plants. Topramezone controls mature *E. indica*, but past efforts to suppress its injury potential to bermudagrass turf have been inconsistent. We hypothesized that metribuzin at 210 g ai ha⁻¹ in admixture with topramezone would improve bermudagrass tolerance while conserving mature *E. indica* control. In preliminary field studies, metribuzin mixed with topramezone at 1.2 or 2.5 g ae ha⁻¹ applied twice at a 3-wk interval reduced bermudagrass injury and white discoloration compared to topramezone applied alone, but did not alter bermudagrass response to mesotrione. Topramezone at 3.7 g ha⁻¹ plus 210 g ha⁻¹ metribuzin

applied twice at a 3-wk interval offered improved bermudagrass tolerance while it still controlled mature *E. indica* during fifteen field and two greenhouse studies in Virginia. This program offered a 10-fold decrease in suprathreshold duration of white-discoloration compared to topramezone alone at 6.1 g ha⁻¹. Bermudagrass absorbed three times less radioactivity than *E. indica* at timings up to 48 h after treatment with ¹⁴C-topramezone. Bermudagrass also metabolized twice as much topramezone compared to *E. indica* at 48 hr after treatment.

Metribuzin reduced ¹⁴C absorption by approximately 25% in both species. These studies confirm the performance of a novel, low-dose topramezone plus metribuzin program for mature *E. indica* control in bermudagrass turf, and suggest that selectivity between bermudagrass and *E. indica* to topramezone is due to differential absorption and metabolism. The fact that metribuzin reduces topramezone absorption in both species suggests that it may help reduce bermudagrass phytotoxic response to topramezone, but its role in altering selectivity between bermudagrass and *E. indica* may be due to other factors.

Keywords:

Days over injury threshold; digital image analysis; topramezone absorption, translocation, and metabolism; turf dark green color index; turfgrass; white discoloration

Introduction

Increased incidences of goosegrass [Eleusine indica (L.) Gaertn.] populations that have developed resistance to effective preemergence herbicides, such as prodiamine and oxadiazon (Breeden et al. 2017; McCullough et al. 2013; McElroy et al. 2017), have forced turfgrass managers to rely on postemergence herbicides for *E. indica* control. Options for postemergence control of *E. indica* in bermudagrass [Cynodon dactylon (L.) Pers.] have become limited due to restrictions on MSMA use and loss of diclofop (Keigwin 2013; McCullough 2014). The loss of these herbicides has led turfgrass managers to increase reliance on products that contain foramsulfuron or metribuzin. Unfortunately, foramsulfuron is both expensive and provides inadequate control of mature *E. indica*, while metribuzin can be highly injurious to bermudagrass at effective rates (Busey 2004, Johnson 1980).

Topramezone is a newer 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibiting herbicide highly efficacious on *E. indica*, with control being observed at one-quarter the labeled rate (Cox et al 2017). In 2018, topramezone was registered for use in bermudagrass for *E. indica* control at rates between 12.3 and 18.5 g ae ha⁻¹, which is half the labeled rate in most coolseason turfgrasses (Anonymous 2018). Even at these lower topramezone rates, bermudagrass is still subject to severe phytotoxicity in the form of foliar bleaching that can persist for multiple weeks (Brewer et al. 2016; Cox et al. 2017; Elmore et al. 2011b). This injury tends to last longer in the transition zone than areas further south due to the shorter growing season and lower accumulation of heat units (Cox et al. 2017; Breeden et al. 2017; Kerr et al. 2019b; Lindsey et al. 2019; Stanford et al. 2005). Topramezone would be a more viable *E. indica* control option for turfgrass managers in the transition zone if programs could be developed to reduce foliar bleaching and injury duration to bermudagrass while also maintaining *E. indica* control efficacy.

In recent years, multiple researchers have evaluated different fertility, herbicide, and irrigation programs with topramezone to reduce bermudagrass phytotoxicity. Triclopyr applied at 140 g ae ha⁻¹ reduced topramezone bleaching injury on bermudagrass while it also maintained adequate *E. indica* control; however, the bermudagrass bleaching injury is replaced with unacceptable leaf necrosis and stunting that persisted far longer than topramezone applied alone (Boyd et al. 2020b; Cox et al. 2017). Iron products significantly reduced bermudagrass bleaching caused by topramezone without compromising *E. indica* control (Boyd et al. 2020a, 2020b). Researchers have also observed that the addition of irrigation immediately after topramezone applied alone at 12.3 g ha⁻¹ or in combination with metribuzin at 420 g ai ha⁻¹ significantly reduced bermudagrass injury from 53 and 82%, respectively, to 11 and 22% one week after initial treatment (WAIT) (Kerr et al. 2019b). During the same studies, topramezone applied alone and in combination with metribuzin controlled mature *E. indica* approximately 50% less when irrigation was applied, but these treatments were not affected when *E. indica* was three tillers or less (Kerr et al. 2019b).

Another potential tank mix partner for topramezone is metribuzin, which is a photosystem II (PS2) inhibitor. Research in disparate agronomic systems, such as turfgrass and production crops, has shown that combinations of HPPD- and PS2-inhibiting herbicides synergized one another for increased weed control (Abendroth et al. 2006; Brosnan et al. 2010; Elmore et al. 2013; Kohrt and Sprague 2017). Currently, there are three papers that have been published evaluating topramezone plus metribuzin in turfgrass. Two evaluated the use of topramezone with or without metribuzin for common bermudagrass suppression and *E. indica* control in seashore paspalum (*Paspalum vaginatum* Sw.) (Lindsey et al. 2019, 2020). They observed that topramezone at 10 g ha⁻¹ plus metribuzin at 100 g ha⁻¹ controlled *E. indica* 90 to

100% (Lindsey et al. 2020). Kerr et al. (2019a, 2019b) sought to reduce bermudagrass response to topramezone at 12 g ha⁻¹ with or without metribuzin at 420 g ha⁻¹ by immediate post-treatment irrigation. Irrigation at 0.6 cm within 1 minute of spray application did not reduce initial bermudagrass injury but slightly improved recovery as assessed at four WAIT in South Carolina and Alabama (Kerr et al. 2019a). In another study, immediate irrigation substantially reduced initial bermudagrass injury but also reduced mature *E. indica* control to less than half of that without irrigation (Kerr et al. 2019b). Kerr et al. (2019b) also noted that topramezone at 12.3 g ha⁻¹ alone controlled mature *E. indica* 66% and control was increased to 100% when an admixture of metribuzin at 420 g ha⁻¹ was added.

These previous studies suggest that metribuzin admixtures to topramezone can improve weed control compared to topramezone alone. We hypothesized that 210 g ha⁻¹ metribuzin as an admixture may allow topramezone rates to be lowered compared to previous work, thus gaining bermudagrass safety while maintaining acceptable *E. indica* control. Our objectives, with respect to this hypothesis, were to (1) evaluate multiple topramezone rates alone or with metribuzin compared to similar programs with mesotrione or sulfentrazone, (2) evaluate a more refined selection of topramezone rates alone or with metribuzin for response of bermudagrass at eight sites and *E. indica* at nine sites. We further desired to elucidate the role metribuzin plays in physiological response of bermudagrass and *E. indica* to topramezone. Previous research has suggested that metabolism might play a role in topramezone selectivity between creeping bentgrass (*Agrostis stolonifera* L.) and targeted weeds (Elmore et al. 2015). Grossman and Ernhardt (2007) showed that selectivity between corn (*Zea mays* L.) and giant foxtail (*Setaria faberi* Herrm.) was primarily based on metabolism. We hypothesized that metabolism may also be involved in selective responses between bermudagrass and *E. indica* and that metribuzin may

alter either absorption, translocation, or metabolism, thus altering plant response. Our final objective (3) was to evaluate absorption, translocation, and metabolism of topramezone in bermudagrass and *E. indica* as influenced by metribuzin admixture.

Materials and methods

Preliminary Experiment Assessing Rates of Topramezone and Mesotrione

Between 2016 and 2020, field experiments were established as randomized complete block designs with four replications and 0.9-by-1.8-m plots to evaluate bermudagrass and smooth crabgrass [Digitaria ischaemum (Schreb.) Schreb. ex Muhl.] response to low rates of mesotrione and topramezone compared to sulfentrazone applied in combination with metribuzin. Bermudagrass tolerance was assessed on research fairways at locations 6 and 9 (Table 1) and D. ischaemum control was assessed at locations 20 and 21 (Table 1). Plant composition and soil edaphic variables for all locations can be referenced in Table 1. Both sites were fertilized a week prior to trial initiation with 24.4 kg N ha⁻¹ and no other fertility or plant protectants were used during the trials. Turf and weedy fallow sites were mown three times per week with reel mowers and irrigation was provided as needed to supplement natural rainfall in order to maintain active turfgrass and weed growth.

Treatments for these trials are shown in Table 2 and included: topramezone (Pylex®, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709, USA), mesotrione (Tenacity®, Syngenta Crop Protection LLC, P.O. Box 18300, Greensboro, NC 27419-8300, USA), and sulfentrazone (Dismiss®, FMC Corporation, 1735 Market Street, Philadelphia, PA 19103) applied alone or mixed with metribuzin (Sencor®, Bayer Environmental Science, A Division of Bayer Crop Science LP, 5000 CentreGreen Way, Suite 400 Cary, NC 27513, USA)

and compared to metribuzin alone. All rates represent the lowest possible rate that was expected to potentially control *E. indica* when mixed with metribuzin. Topramezone-containing treatments were applied with 0.5% v/v of methylated vegetable oil (MVO) (Dyne-Amic®, Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN 38017, USA), and mesotrione-containing treatments were applied with 0.25% v/v of nonionic surfactant (NIS) (Induce®, Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN 38017, USA). All treatments were applied using a CO₂-pressurized hooded sprayer calibrated to deliver 280 L ha⁻¹ at 289 kPa via two TeeJet XR6502VS flat fan nozzles (TeeJet; Spraying Systems Co., Glendale Heights, IL 62703).

In this preliminary study, turf response and weed control were assessed visually at 0, 1, 2, 4, 5, and 8 WAIT while *D. ischaemum* cover was based on line intersect counts using a 0.91-m-by-0.91-m grid that contained 240 intersects at 5.72-cm increments at 8 WAIT. Turf injury and weed control were estimated as percentage loss of perceived green vegetation potential based on nontreated turf (Frans et al. 1986). Turf white discoloration was estimated as the percentage of turfgrass foliage that exhibited white discoloration. The degree to which turfgrass injury and discoloration are objectionable to turf managers is temporally dependent. Longer durations of turf discoloration are generally unacceptable. To assess duration of objectionable injury and white discoloration, data were converted to days over a threshold (DOT) by assuming linear trends between assessment dates and using functional arguments in Microsoft Excel software (Microsoft Excel®, Microsoft Corporation, Redmond, WA 98052) to calculate the number of days that estimated injury or discoloration was above a threshold of 30 and 10%, respectively. These metrics, expressed as DOT₃₀ and DOT₁₀, reflect the amount of time that turfgrass injury and white discoloration were above acceptable levels as has been done in other studies (Cox et

al. 2017). In addition to injury duration, maximum injury is also of concern and was calculated by recording maximum observed injury values from each experimental unit over the span of assessment dates.

Turf injury maxima, turf injury DOT₃₀, Turf white discoloration DOT₁₀, and D. ischaemum cover and control 8 WAIT were subjected to a combined analysis of variances (ANOVA) with sums of squares partitioned to reflect effects of rep, trial, treatment, and trial by treatment (McIntosh 1983). Trial was considered a random variable and mean squares of treatment effects were tested by the mean square associated with trial by treatment. If trial by treatment interactions were significant, data were separated by trial, otherwise data were averaged over trial. Means were separated using Fisher's Protected LSD test at $\alpha = 0.05$.

Performance of Selected Topramezone + Metribuzin Programs at Multiple Sites

Fifteen field and two greenhouse studies were conducted as randomized complete block designs with four replications to investigate *E. indica* control and bermudagrass response to topramezone plus metribuzin programs. Bermudagrass response was assessed at locations 1, 2, 3, 4, 5, 7, 8, and 10 while *E. indica* response was assessed at locations 11, 12, 13, 14, 15, 16, 17, 18, and 19 (Table 1). Plant composition and soil edaphic variables for all locations can be referenced in Table 1. All field locations were fertilized monthly during the growing season to provide 49 kg N ha⁻¹ and irrigated as needed to maintain active turfgrass and weed growth. All bermudagrass turf was mown three times per week with reel mowers at heights shown in Table 1. Fallow, weedy locations were mown weekly or biweekly with reel mowers for location 18 and rotary mowers for all other locations. In greenhouse locations 11 and 12, *E. indica* was clipped with scissors twice per week to maintain an approximate height of 7.6 cm and pots were irrigated daily. Green house pots were 10.2-cm diameter and filled with a soil and sand (2:1 by wt)

mixture supplemented with 25 kg N ha⁻¹ monthly. The soil information can be found in Table 1. Supplemental lighting provided approximately 530 µmol m⁻² s⁻¹ photosynthetically active radiation via high-pressure sodium lamps for 14 hr each day and plants were maintained at 26/24 °C day/night temperatures.

The plot sizes for the bermudagrass tolerance sites were 1.2 m by 1.8 m, while the plot sizes for the *E. indica* control sites ranged from 1.2 m by 1.2 m to 1.8 m by 1.8 m. The plot sizes for weed control sites varied due to *E. indica* pressure and space availability. Treatments included topramezone applied at 1.2, 3.7, and 6.1 g ha⁻¹ plus metribuzin applied at 210 g ha⁻¹, and topramezone at 6.1 g ha⁻¹ applied alone. All treatments included 0.5% v/v of MVO and were applied twice at a 3-wk interval. Greenhouse pots were sprayed using a CO₂-pressurized spray chamber that deliver 280 L ha⁻¹ at 289 kPa via one Teejet 80015 even flat fan nozzle. Field plots were sprayed using a CO₂-pressurized sprayer with two Teejet TTI 11003 nozzles or four Teejet TTI 11004 nozzles that delivered 280 L ha⁻¹ at 289 kPa.

Turf response was evaluated at 0, 1, 2, 3, 4, 5, 6, and 8 WAIT. Turf injury maxima, injury DOT₃₀ and white discoloration DOT₁₀ were assessed as previously described. Turf dark green color index (DGCI) and green cover was assessed via analysis of aerial images in Field Analyzer (Turf Analyzer; Fayetteville, AR 72701) with selected settings of low hue from 70 to 80, high hue at 360, low saturation from 29 to 38, high saturation at 100, low brightness at 0, and high brightness from 60 to 68. Grid settings included an X-offset of 20 and Y-offset of 20 to reduce any variable edge effect caused by incorrect sprayer overlap. Normalized Difference Vegetation Index (NDVI) was also collected at the tolerance sites at 1, 2, 4, 5, 6 and 8 WAIT using a multispectral analyzer (Crop CircleTM Model ACS-210, Holland Scientific Inc., 6001 South 58th Street, Lincoln, NE 68516). At the *E. indica* control sites, visual percent cover and control were

visually assessed (0-100% scale) at 0, 1, 2, 3, 4, 6, and 8 WAIT, and final plant counts were taken at 8 WAIT. In the two greenhouse trials, *E. indica* foliar biomass was also assessed at 9 WAIT by cutting all foliage at ground level, drying at 50 °C for 48 hours, and weighing. Data were subjected to ANOVA and mean separation as previously described.

Absorption, translocation and metabolism of ¹⁴C-topramezone

Premier Pro brand (variety name is Premier) bermudagrass sprigs were removed from the field and planted into sand flats in addition to *E. indica* seed that had been collected from a local turf research area. Both the *E. indica* and bermudagrass transplants were treated with fluxapyroxad plus pyraclostrobin and 49 kg N ha⁻¹ to help maintain plant health and reduce disease occurrence. Once *E. indica* matured to a three-leaf stage and bermudagrass was producing new shoots and leaves, plants were selected for size consistency and carefully removed from the flat, rinsed, and transplanted into 50-ml centrifuge tubes with a Hoagland modified basal salt solution (MP Biomedicals, 29525 Foundation Pkwy, Solon, OH 44139) mixed at 50% strength and the plants were held in place by cotton balls. Plants were maintained in hydroponic culture for approximately one week in controlled-environment chambers (350 µmol m⁻² sec⁻¹ PAR for 12 h at 26/20 °C day/night temperatures, respectively and 40% relative humidity). *Eleusine indica* seedlings had five leaves and bermudagrass sprigs had a single shoot with six expanded leaves at application time.

Treatments were arranged in a split-split-plot design containing four harvest times as main plots, a two by two factorial arrangement containing two plant species (bermudagrass vs *E. indica*) and two herbicide treatments (topramezone vs topramezone + metribuzin) as sub plots, and five plant partitions as sub-sub plots. The study was repeated in two separate growth chambers with both studies initiated on January 3, 2021. The two herbicide solutions consisted

of [phenyl-U-¹⁴C] topramezone (96% radiochemical purity, 4.14 MBq mg⁻¹) dissolved in water and formulated topramezone product (Pylex SC) with 5% v/v MVO or topramezone and formulated metribuzin product (Sencor 75DF) with MVO. For these herbicide solutions, we followed procedures similar to Grossman and Ehrhardt (2007), but used lower topramezone field rates. The ratio of formulated topramezone and metribuzin, water, and MVO were equivalent to that applied in the field studies when topramezone was applied at 3.7 g ha⁻¹, metribuzin was applied at 210 g ha⁻¹, the water volume was 280 L ha⁻¹, and MVO was included at 0.5% v/v. Both *E. indica* and bermudagrass received two 1 μl droplets of solution applied via microsyringe to the adaxial surface of the third-newest, fully expanded leaf equaling 7.5 kBq plant⁻¹.

The treated plants were harvested at 0.25, 5, 24, and 48 hours after treatment (HAT). At each harvest time, the treated leaf was excised and vortexed in cold 1:1 methanol:deionized water with 1% v/v MVO once for 60 seconds. Once the leaf wash was complete, the treated leaf, all foliage above the treated leaf, all foliage below the treated leaf, and roots were placed into a freezer at -18 °C to await further processing. For extraction, tissue samples were removed from the freezer and macerated in 6 ml of cold methanol with a glass tissue grinder (PyrexTM Glass Pestle Tissue Grinders, Corelle Brands, LLC, Rosemont, IL 60018). The ground tissue and extraction solution were then vacuum filtrated using a Buchner funnel and 55 mm filter paper (WhatmanTM Filter Paper Grade 1, Cytiva Life Sciences, Marlborough, MA 01752). All glassware was rinsed into the filtration apparatus with an additional 4 ml of methanol. A 0.5 ml aliquot of each extraction solution, the rinse solution, and the nutrient solution (to assess root exudate) was placed into separate 20-ml glass vials with 15 ml scintillation cocktail (ScintiVerse® BD, Fisher Scientific, Fair Lawn, NJ 07410) and radioactivity was determined

using a liquid scintillation spectrometer (LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, Inc., Fullerton, California 92634-3100, USA).

Radioactivity extracted from treated leaves after 48 hr was partitioned using thin-layer chromatography. Homogenates from previously described extraction and filtration procedures were dried in a nitrogen evaporator (N-EVAPTM 112, Organomation Associates Inc., Berlin, MA 01503) and residues were resuspended with 100 µl cold methanol. This resuspended solution was then delivered to a 20 cm by 20 cm silica gel thin layer chromatography (TLC) plate (TLC Silica gel 60G F₂₅₄, Millipore Sigma, Burlington, MA 01803) and developed in a 3:2 v/v solution of cold ethyl acetate: methanol within an airtight glass chamber. The plates were then air dried and radioactive positions, proportions, and corresponding R_f values were determined with a radiochromatogram scanner (Bioscan, System 200 Imaging Scanner and Auto Changer 1000, Bioscan Inc., Washington, DC 20007). Parent herbicide was identified by comparing with radiolabeled standards spotted on adjacent lanes of each plate. Radioactive trace peaks were integrated with Win-Scan software (WIN-SCAN Imaging Scanner Software Version 1.6c, Bioscan Inc., Washington, DC 20007) with smoothing set to 13 point cubic and background excluded from peak area calculation. Area under each peak was converted to a percentage of total area and expressed as herbicide, more-polar metabolites, and less-polar metabolites.

Data consisted of extracted ¹⁴C radioactivity from rinse, treated leaf, above treated leaf, below treated leaf, roots, and nutrient solution at four harvest times. Total absorbed radioactivity was computed as the sum of radioactivity counts from all samples except the rinse and these were expressed as a percentage of recovered herbicide. Radioactivity extracted from specific plant parts at 24 and 48 HAT were expressed as percentage of absorbed radioactivity. Radioactivity extracted from treated leaves at 48 HAT were expressed as the percentage of all

peak areas below (more polar) and above (less polar) the topramezone peak compared to the percentage area under the peak identified as topramezone. All data were subjected to ANOVA with sums of squares partitioned to reflect the split-split plot treatment structure and the random variable trial. All main effects or interactions of fixed effects were tested by the mean square associated with their interaction with trial. If trial interaction was significant, effects were presented separately by trial, otherwise, significant effects or interactions were averaged over trial.

Results and Discussion

Preliminary Experiment Assessing Rates of Topramezone and Mesotrione

There was a significant trial by treatment interaction for turf injury maxima and turf injury DOT₃₀ (P < 0.0001), so these response variables were separated by trial (Table 2). Turfgrass white DOT₁₀ and *D. ischaemum* control and cover had a significant treatment effect (P < 0.0001) that was not dependent on trial (P \geq 0.1023), so data were averaged across trials (Table 2). The trial interaction for turf injury maxima was likely caused by inconsistent injury response to all treatments except topramezone plus metribuzin (Table 2). At location 6 (see Table 1), 'Premier Pro brand' bermudagrass was more injured by most treatments than the 'Tifway 419' bermudagrass at location 9. Bermudagrass varieties have been shown to similarly differ in response to topramezone (Cox et al. 2017) and 'Tifway 419' has been shown to tolerate mesotrione more than some other bermudagrass varieties (Elmore et al. 2011a, 2011b).

The addition of metribuzin to either rate of topramezone effectively reduced maximum injury to near or below an acceptable injury level ($\leq 35\%$) compared to 52 to 69% injury by topramezone alone depending on trial (Table 2). Metribuzin did not improve maximum injury

response by mesotrione, which was 58 to 93% depending on treatment and location. Investigation of topramezone at these rates on bermudagrass safety or *D. ischaemum* control have not been previously reported. Lindsey et al. (2019, 2020) observed that topramezone applied at 5 to 20 times higher rates than the current study and mixed with metribuzin injured bermudagrass greater than 50%, but the bermudagrass recovered to an acceptable injury level (≤ 30%) after 7 days in Hawaii. Only one study has evaluated mesotrione in combination with metribuzin at similar rates as the current study. Lindsey et al. (2019) observed that mesotrione at 67 g ai ha⁻¹ plus metribuzin at 100 g ha⁻¹ injured bermudagrass less than 10% in a greenhouse study in Hawaii, which is significantly less than the current study. Brewer et al. (2016) observed that mesotrione applied at 280 g ha⁻¹ injured bermudagrass 66 and 83%, which is similar injury results as the current study but 2 to 4 times the mesotrione rate.

The turf injury DOT₃₀ was dependent on trial location for the same reason as turf injury maxima (Table 2). Topramezone at 1.2 g ha⁻¹ plus metribuzin had only 0 to 0.5 DOT₃₀ depending on location and less than all other treatments except metribuzin at both locations and sulfentrazone plus metribuzin at location 6 (Table 2). Topramezone applied alone at 2.5 g ha⁻¹, by comparison, injured bermudagrass over the 30% threshold for 10 to 16 days depending on location. This dramatic difference in both magnitude of injury and recovery time when metribuzin was mixed with low-dose topramezone treatments was the observation that stimulated the other research in this report. The primary reason that injury levels were substantially reduced when 210 g ha⁻¹ metribuzin was added to topramezone has to do with reduced white tissue discoloration.

Turf white tissue discoloration DOT_{10} was consistent between trials and eliminated by adding metribuzin to topramezone. This combination did not result in any days over a 10%

threshold of white discoloration to bermudagrass foliage compared to 19 days from topramezone alone (Table 2). Metribuzin also reduced white discoloration DOT₁₀ when added to mesotrione compared to mesotrione alone despite overall injury from these treatments being unacceptable.

Digitaria ischaemum was controlled best by mesotrione programs or sulfentrazone plus metribuzin (Table 2). Topramezone alone or admixture did not control *D. ischaemum* more than 46% despite an improvement when metribuzin was an admixture. Trends in *D. ischaemum* cover mirrored that of *D. ischaemum* control (Table 2). Similar to the current study, Elmore et al. (2012) observed that mesotrione applied once at 140 g ha⁻¹ controlled *D. ischaemum* between 70 and 80%, while topramezone applied once at 4.5 g ha⁻¹ controlled *D. ischaemum* less than 10%. At this time, no research has been published evaluating combinations of mesotrione or topramezone with metribuzin for *D. ischaemum* control. The lack of *D. ischaemum* control in the preliminary study was of little consequence since our objective was to develop programs for selective *E. indica* control.

Performance of Selected Topramezone + Metribuzin Programs at Multiple Sites

Bermudagrass response. Due to results from the preliminary experiment, three low-dose topramezone treatments mixed with metribuzin and compared to topramezone alone at 6.1 g ha⁻¹ were evaluated at multiple sites to assess consistency of bermudagrass response and *E. indica* control efficacy. All response variables associated with bermudagrass response and *E. indica* control, except *E. indica* biomass, had a significant trial by treatment interaction that we believe is due to the sheer number of study locations involved and is of limited biological significance (Table 3). It was noted that the F-values of the main effects when tested by the mean square error of each main effect's interaction with trial, was always at least four and usually greater than ten orders of magnitude higher than that of the interaction of each effect with trial (Table 3). These

trends in F-values suggest that the properly tested main effects account for considerably more variance than the trial interactions. Upon investigating the likely reason for the trial interactions, we discovered that small deviations associated with the lowest two topramezone rates caused these two treatments to sometimes differ and sometimes be equivalent (data not shown). Such deviations in mean rank never occurred in more than two of the eight trials and were associated with low-level responses (e.g., 25% versus 35%). For these reasons and to reduce presented data by eight orders of magnitude, we decided to present the treatment main effects rather than the trial interactions but we included the standard errors for each mean as a demonstration of consistency across trials (Table 4).

Turfgrass injury maxima increased stepwise between 24 and 74% as topramezone rate increased following two topramezone plus metribuzin treatments at 3-wk intervals (Table 4). All treatments with metribuzin admixture had less maximum injury than topramezone alone at 6.1 g ha⁻¹. As of this writing, there are only three published experiments that evaluated combinations of topramezone with metribuzin (Kerr et al. 2019b; Lindsey et al. 2019, 2020), but none evaluated topramezone rates as low as the current trials. Lindsey et al. (2019, 2020) evaluated topramezone at 10 to 12 g ha⁻¹ with 100 g ha⁻¹ metribuzin on seashore paspalum turf in Hawaii. Kerr et al. (2019b) evaluated topramezone at 12 g ha⁻¹ mixed with metribuzin at 420 g ha⁻¹ on bermudagrass turf in South Carolina. Both researchers observed bermudagrass injury of greater than 50%. The maximum injury observed in the current trial is similar to maximum bermudagrass injury observed by Cox et al. (2017) when topramezone was applied alone at 6.1 and 12 g ha⁻¹.

Turf injury DOT₃₀ was 0.56 and 7.5 days following two treatments of topramezone at the two lowest rates mixed with metribuzin (Table 4). Two applications of topramezone at 6.1 g ha⁻¹

injured bermudagrass more than 30% for 20 days when mixed with metribuzin and 30 days when applied alone. Topramezone applied twice at 1.2 and 3.7 g ha⁻¹ with metribuzin caused bermudagrass white discoloration above 10% for 0 and 3.8 days (Table 4). When the topramezone rate was increased to 6.1 g ha⁻¹, bermudagrass white discoloration exceeded 10% for 16 days when metribuzin was added and 30 days when topramezone was applied alone. Cox et al. (2017) observed that topramezone applied twice (3-wk interval) at either 6.1 or 12 g ha⁻¹ resulted in bermudagrass white discoloration DOT₁₀ between 25 to 40 days and a bermudagrass injury DOT₃₀ between 19 and 30 days across 31 bermudagrass varieties maintained at fairway height of cut in Virginia.

The DGCI AUPC d⁻¹ and the digitally-analyzed green cover AUPC d⁻¹ both exhibit stepwise reductions that mirror the stepwise increase in injury responses (Table 4). The DGCI data were presented instead of NDVI since trends for both followed closely to one another. Since all herbicide treatments caused at least some reduction in DGCI and green turf cover, practitioners should expect some level of decline in turfgrass aesthetics. The combinations of metribuzin with topramezone at 1.2 and 3.7 g ha⁻¹ have DGCI and green cover that is close enough to nontreated turf to suggest that turf aesthetic decline is of low magnitude and transient, and this assumption is supported by the maximum injury, injury DOT₃₀, and white discoloration DOT₁₀ data (Table 4).

These data show that metribuzin substantially lowers both magnitude and duration of turf injury and white discoloration while improving bermudagrass DGCI and green cover. The most striking reduction in bermudagrass response, however, requires lowering the topramezone rate to at least 3.7 g ha⁻¹ along with the metribuzin admixture. These safety margins of 4- to 10-fold by

programs that include topramezone at or below 3.7 g ha⁻¹ compared to topramezone alone at 6.1 g ha⁻¹ are of little use if they do not control mature E. *indica*.

Goosegrass (Eleusine indica) control. During the field trials, we rarely observed any treatment controlling *E. indica* 100% due to occasional plant survival and subsequent seedling germination that occurred near the final assessment. None of the treatments seemed to have significant pre-emergent suppression of *E. indica*. When averaged over nine site years, two applications of topramezone at 3.7 g ha⁻¹ plus metribuzin controlled *E. indica* 94% and equivalent to two applications of topramezone applied at 6.1 g ha⁻¹ alone (Table 5). When the topramezone rate was dropped to 1.2 g ha⁻¹, *E. indica* control fell to 80%. The same trends were evident for *E. indica* cover reduction, plant density, and foliar biomass (Table 5). Kerr et al. (2019b) and Lindsey et al. (2020) both observed that higher rates of topramezone plus metribuzin controlled *E. indica* 80 to 100%, which is similar to the results from the lower topramezone rates in the current study.

The results of these studies suggest that two applications of topramezone at 3.7 g ha⁻¹ plus metribuzin at 210 g ha⁻¹ is an optimal program for selective *E. indica* control in bermudagrass turf. This program will cause transient injury to bermudagrass but dramatically reduces recovery time such that the duration of objectionable turfgrass aesthetics is minimized. Our studies align with observations by other researchers that metribuzin admixture with topramezone can increase *E. indica* control. Further studies were conducted to elucidate the mechanism behind this interaction.

Absorption, translocation and metabolism of ¹⁴C-topramezone

Recovered radioactivity was $93 \pm 8\%$ from the 64 plants treated in this study (data not shown). The harvest time by species interaction (Figure 1A) and the herbicide main effect (Figure 1B) were significant for total absorbed radioactivity (P < 0.05). *Eleusine indica* absorbed three times as much radioactivity as bermudagrass within 48 hr following treatment of either ¹⁴Ctopramezone alone or ¹⁴C-topramezone plus metribuzin (Figure 1A). This separation was evident even at 15 minutes after treatment, suggesting that bermudagrass may absorb topramezone more slowly than E. indica. Eleusine indica, unlike bermudagrass, also did not appear to have reached an asymptote for ¹⁴C absorption by 48 HAT, suggesting additional herbicide absorption may have occurred with more time. We chose the maximum harvest time of 48 HAT based on work by Grossman and Ernhardt (2007) that showed no additional absorption in corn or giant foxtail between 24 and 48 HAT. The level of absorption observed in both bermudagrass and E. indica is considerably lower than the amount of radioactivity that Grossman and Ernhardt (2007) extracted from corn and giant foxtail. This disparity in absorption rates could be due to a 20-fold increase in the amount of formulated product used by Grossman and Ernhardt (2007) in their spotting solution. Since our objective was to compare topramezone alone to topramezone plus metribuzin, it was extremely important that we replicate the ratios of water, topramezone, metribuzin, and adjuvant that was used in our field studies.

The addition of metribuzin to ¹⁴C-topramezone decreased absorption consistently at all harvest times and average ¹⁴C radioactivity recovered from plants across all times and both species was 12% when ¹⁴C-topramezone was applied alone and 9% when ¹⁴C-topramezone was mixed with metribuzin (Figure 1B). This 25% reduction in ¹⁴C absorption could partially explain the reduced bermudagrass injury that has been observed in our field studies when metribuzin is mixed with topramezone.

The interaction of sample by herbicide by species was significant (P < 0.05) for percentage of absorbed radioactivity extracted from plants at 24 and 48 HAT (Table 6). At 24 HAT, twice as much absorbed radioactivity translocated out of treated bermudagrass leaves when topramezone was applied alone compared to when mixed with metribuzin. The same trend was not evident in *E. indica*. Thus, metribuzin may partially protect bermudagrass from the injurious effects of topramezone via altered translocation. At 48 HAT, 51% of absorbed radioactivity had translocated out of topramezone-treated bermudagrass leaves compared to only 18% translocation following treatment of topramezone plus metribuzin (Table 6). Metribuzin had a similar inhibitory effect on 14 C translocation in *E. indica*, but at a smaller magnitude. It is also possible that these changes in 14 C translocation may be of no consequence as the translocated radioactivity could be a metabolite of topramezone rather than the active ingredient.

The main effect of species was significant for percentage proportions of radioactivity between metabolites and parent herbicide (P < 0.0001) and not dependent on trial or herbicide (P > 0.05). At 48 HAT, bermudagrass had metabolized 38% of absorbed radioactivity in treated leaves compared to only 20% metabolism by *E. indica* (Table 6). Bermudagrass had a greater percentage of polar metabolites compared to nonpolar metabolites while the two were equivalent in *E. indica*. Based on previous reports, bermudagrass metabolizes topramezone similar to giant foxtail, *E. indica* metabolizes topramezone similar to sorghum (*Sorghum bicolor* L.), and corn metabolizes topramezone more rapidly than all of these species (Grossman and Ernhardt 2007).

These data show that bermudagrass absorbs one-third as much radioactivity following ¹⁴C-topramezone treatment and metabolizes approximately twice as much topramezone compared to *E. indica* in the first 48 HAT. These trends could explain differential response between the two species. These data further suggest that altered absorption and/or translocation

caused by metribuzin admixture could partially explain why bermudagrass injury and recovery time is substantially reduced by said mixture. The mixture of 3.7 g ha⁻¹ topramezone plus 210 g ha⁻¹ metribuzin applied twice was found to control *E. indica* at commercially acceptable levels and equivalent to topramezone at 6.1 g ha⁻¹ while reducing days over a 10% white discoloration threshold nearly 10-fold and reducing days over a 30% injury threshold 4-fold. We also found that metribuzin admixture substantially increases *D. ischaemum* control by topramezone but not to commercially-acceptable levels when topramezone rates are less than 6.1 g ha⁻¹.

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Table 1. Plant composition, edaphic variables, and initial application date for 21 unique site locations in Blacksburg, VA utilized to assess goosegrass [*Eleusine indica* (L.) Gaertn.] or smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.] control or bermudagrass turf tolerance to topramezone-based herbicide programs.

Loca	tion		Bermudagrass	Average					
			variety or weed	bermudagrass	Mowing	Soil	Soil	Soil	
No.	Site	Position	growth stage	or weed cover	height	type ^b	Ph	OM	Initial Application ^c
				%	-cm-			%	
1	GRF^a	37.2339 °N, 80.4358 °W	Latitude 36	98	1.91	S 1	6.1	3.6	Aug 5, 2018
2	GRF	37.2339 °N, 80.4358 °W	Latitude 36	97	1.91	S 1	6.1	3.6	Aug 4, 2019
3	TRC	37.2120 °N, 80.4132 °W	Patriot	96	1.52	S2	5.5	3.9	Aug 4, 2019
4	GRF	37.2339 °N, 80.4369 °W	PremierPRO	98	1.91	S 2	6.6	3.8	Aug 5, 2018
5	GRF	37.2339 °N, 80.4369 °W	PremierPRO	98	1.91	S2	6.6	3.8	Aug 4, 2019
6	GRF	37.2339 °N, 80.4369 °W	PremierPRO	99	1.91	S2	6.6	3.8	Aug 17, 2020
7	GRF	37.2345 °N, 80.4362 °W	Riviera	93	1.91	S2	6.9	4.2	Aug 5, 2018
8	GRF	37.2345 °N, 80.4362 °W	Riviera	95	1.91	S2	6.9	4.2	Aug 4, 2019
9	TRC	37.2118 °N, 80.4129 °W	Tifway 419	97	1.52	S 1	5.0	2.7	July 14, 2016
10	TRC	37.2118 °N, 80.4129 °W	Tifway 419	97	1.52	S 1	5.0	2.7	Aug 4, 2019
11	GRGH	37.2319 °N, 80.4358 °W	3-7 tiller G ^a		7.6	S2	6.3	3.9	July 1, 2016
12	GRGH	37.2319 °N, 80.4358 °W	5-8 tiller G		7.6	S2	6.3	3.9	Nov 11, 2016
13	TRC	37.2146 °N, 80.4127 °W	3-7 tiller G	24	3.8	S 3	6.4	0.9	July 16, 2017
14	TRC	37.2125 °N, 80.4129 °W	4-10 tiller G	40	3.8	S2	6.1	2.9	July 18, 2018
15	TRC	37.2146 °N, 80.4127 °W	4-10 tiller G	72	5.1	S 3	6.4	0.9	Aug 5, 2018
16	VTGC	37.2275 °N, 80.4307 °W	5-11 tiller G	60	5.1	S 2	7.7	4.5	Jun 21, 2019
17	TRC	37.2146 °N, 80.4127 °W	7-12 tiller G	52	3.8	S 3	6.4	0.9	Jul 30, 2019
18	TRC	37.2142 °N, 80.4110 °W	4-13 tiller G	25	1.5	S 4	6.4	1.4	Jul 31, 2019
19	TRC	37.2125 °N, 80.4129 °W	4-15 tiller G	33	3.8	S 2	6.1	2.9	Jul 31, 2019
20	TRC	37.2118 °N, 80.4129 °W	5-15 tiller SC	32	1.5	S 2	5.0	2.7	Jul 14, 2016
21	TRC	37.2118 °N, 80.4129 °W	3-10 tiller SC	25	1.5	S 2	5.0	2.7	Jun 26, 2017

^aAbbreviations: G, goosegrass; GRF, Glade Road Research Facility; GRGH, Glade Road Greenhouse; OM, organic matter; SC, smooth crabgrass; TRC, Turfgrass Research Center; VTGC, Virginia Tech Golf Course.

^bSoil taxonomy: S1- Duffield silt loam (fine-loamy, mixed, active, mesic, Ultic Hapludalfs)-Ernest silt loam (fine-loamy, mixed, superactive, mesic Aquic Fragiudults) complex; S2- Groseclose-Urban land complex loam (clayey, mixed, mesic Typic Hapludults); S3- USGA specification sand; S4- Udorthents and Urban land plus a sand cap.

^cAll sites received sequential applications three weeks after the initial.

Table 2. Preliminary experiment investigating herbicide combinations for effects on bermudagrass injury maxima and days over a threshold of 30% turf injury (DOT₃₀), days over a white discoloration threshold of 10% (DOT₁₀) and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.] control and cover. Data were averaged over two locations (Loc.) if trial by treatment interactions were insignificant (P > 0.05).

	Turf injury maxima Turf injury DOT ₃₀		ary DOT ₃₀	White discoloration	Smooth crabgrass			
Herbicide(s) ^a	Rates ^b	Loc. 6	Loc. 9	Loc. 6	Loc. 9	DOT_{10}	Control	Cover
	g ha ⁻¹	9	% ———		d		%	
Nontreated								56
Topramezone + metribuzin	1.2 + 210	25	30	0	0.5	0	39	23
Topramezone + metribuzin	2.5 + 210	35	28	6.8	0	0	46	24
Topramezone	2.5	69	52	16	10	19	10	39
Mesotrione + metribuzin	70 + 210	78	58	19	14	1.0	98	1.8
Mesotrione + metribuzin	140 + 210	93	72	25	19	7.1	99	0.2
Mesotroine	140	84	68	22	15	24	95	3.8
Sulfentrazone + metribuzin	280 + 210	39	52	1.1	4.6	0	89	7.2
Metribuzin	210	15	27	0	0.7	0	48	31
LSD (0.05)		4.1	^a 9.3	1.9	2.3	2.6	9.9	12

^aAll treatments were applied twice at a 3-week interval.

^bRates given as acid equivalency for topramezone and active ingredient for all other herbicides.

Table 3. Analysis of variance for nine response variables showing summary statistics for treatment main effects and trial by treatment interactions.

Dependent variable	Tre	atment	Trial x Treatment		
	F value	Pr > F	F value	Pr > F	
Bermudagrass injury maxima	174	< 0.0001	4.87	< 0.0001	
Bermudagrass injury DOT ₃₀ ^a	137	< 0.0001	4.71	< 0.0001	
Bermudagrass white discoloration DOT ₁₀	68.5	< 0.0001	17.7	< 0.0001	
DGCI ^a AUPC d ⁻¹	30.3	< 0.0001	2.68	< 0.0001	
Turf green cover AUPC d ⁻¹	72.5	< 0.0001	3.19	< 0.0001	
Goosegrass control 8 WAIT ^a	259	< 0.0001	4.69	< 0.0001	
Goosegrass cover reduction	92.1	< 0.0001	5.06	< 0.0001	
Goosegrass plant density	38.1	< 0.0001	4.36	< 0.0001	
Goosegrass biomass	70.8	< 0.0001	2.79	0.3658	

^aAbbreviations: AUPC, area under the progress curve; DGCI, dark green color index; DOT₁₀, days over a threshold of 10%; DOT₃₀, days over a threshold of 30%; WAIT, weeks after initial treatment.

Table 4. Influence of herbicide treatment on bermudagrass injury maxima, days over an injury threshold of 30% (DOT₃₀), days over a white discoloration threshold of 10% (DOT₁₀), average dark green color index (DGCI) d^{-1} based on area under the progress curve (AUPC d^{-1}), digital-image-assessed percentage turf green cover AUPC d^{-1} mean \pm SE over eight site years.

Herbicide(s) ^a	Rate ^b	Turf injury maxima	Turf injury DOT ₃₀	Turf white DOT ₁₀	DGCI AUPC d ⁻¹	Green cover AUPC d ⁻¹
	g ha ⁻¹	%		d	——index ——	%
Nontreated					0.606 ± 0.036	87 ± 1.5
Topramezone + metribuzin	1.2 + 210	24 ± 1.9	0.56 ± 0.30	0 ± 0	0.585 ± 0.035	80 ± 2.8
Topramezone + metribuzin	3.7 + 210	45 ± 4.2	7.5 ± 1.4	3.8 ± 2.2	0.564 ± 0.031	76 ± 1.9
Topramezone + metribuzin	6.1 + 210	74 ± 3.2	20 ± 1.2	16 ± 2.0	0.540 ± 0.029	67 ± 1.6
Topramezone	6.1	89 ± 1.6	30 ± 1.8	31 ± 1.8	0.520 ± 0.023	52 ± 2.6
LSD (0.05)		2.8	1.4	1.1	0.011	2.5

^aAll treatments were applied twice at a 3-week interval.

^bRates given as acid equivalency for topramezone and active ingredient for metribuzin.

Table 5. Influence of herbicide treatment on goosegrass [*Eleusine indica* (L.) Gaertn.] control, cover reduction, and plant density m^{-2} as mean \pm SE over nine site years and plant biomass pot⁻¹ averaged over two greenhouse trials.

	Goosegrass							
Herbicide(s) ^a	Rate ^b	Control	Cover reduction	Plant density	Biomass			
	g ha ⁻¹		- %	—no. m ⁻² —	—_ g pot ⁻¹ —			
Nontreated			0.4 ± 0.3	78 ± 13	1.2			
Topramezone + metribuzin	1.2 + 210	80 ± 3.6	71 ± 5.8	18 ± 5.1	0.19			
Topramezone + metribuzin	3.7 + 210	94 ± 1.6	89 ± 2.2	8.3 ± 3.4	0.27			
Topramezone + metribuzin	6.1 + 210	98 ± 0.7	97 ± 0.7	4.4 ± 2.7	0.21			
Topramezone	6.1	97 ± 0.8	95 ± 0.9	5.6 ± 3.0	0.19			
LSD (0.05)		3.4	6.2	6.8	0.15			

^aAll treatments were applied twice at a 3-week interval.

^bRates given as acid equivalency for topramezone and active ingredient for metribuzin.

Table 6. Percentage of absorbed radioactivity extracted from bermudagrass and goosegrass [*Eleusine indica* (L.) Gaertn.] tissue and nutrient solution at 24 and 48 hours after treatment (HAT) and percentage of radioactivity traces from thin-layer chromatographic separations of parent herbicide and polar/nonpolar metabolites from treated leaves 48 hr following ¹⁴C-topramezone or ¹⁴C-topramezone plus metribuzin treatment to the adaxial surface of the third newest leaf. Translocation data are averaged over trial and metabolism data are averaged over trial and herbicide mixture.

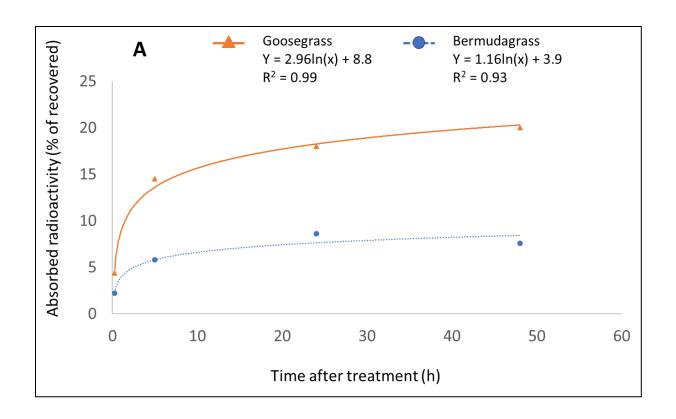
	F	Bermudagrass		Goosegrass					
	Topramezone	Topram ^a + metr	ibuzin	Topramezone		am + metribuzin			
24 HAT ^a									
Treated leaf (TL)	$60*+^{b}$	80*+		89*+		89*+			
Above TL	14*+	8*+		3.5*+		4.1*+			
Below TL	21*+	10*+		5.5*+		5.1*+			
Root	4.9*+	2.2*+		1.8*		1.8*+			
Root Exudate	0.7	0		0.1		0.1			
LSD	3.9	5.6		3.1		3.2			
48 HAT									
Treated leaf (TL)	49*+	82*+		79*+		90*+			
Above TL	27*+	6.5*+		7.8*+		3.9*+			
Below TL	19*+	8*+		10*+		4.5*+			
Root	4.5	2.8		2.7*		1.1*			
Root Exudate	0.2	0.4		0.5		0.1			
LSD	4.1	5.0		3.5		3.6			
	Partitioned radioactivity from bermudagrass			Partitioned radioactivity from gooseg					
	Topramezone	More polar Less	polar T	Горгатеzone	More polar	Less polar			
			% _						
48 HAT									
Treated leaf	62*	23* 14	1*	80*	11*	8.1*			

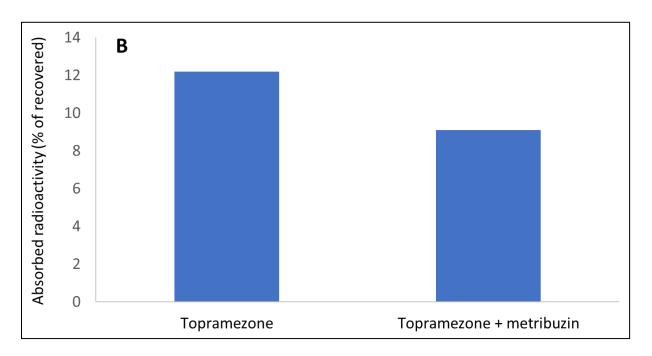
^aAbbreviations: HAT, hours after treatment; TL, treated leaf; Topram, topramezone.

 bA * or a + after a given mean denotes significant difference between herbicide mixtures or species, respectively, based on Fisher's Protected LSD test at P \leq 0.05).

Figure legends.

Figure 1. Percentage of recovered radioactivity over time absorbed by bermudagrass or goosegrass [*Eleusine indica* (L.) Gaertn.] averaged over trial and herbicide mixture (A) and the average absorbed radioactivity as influenced by herbicide averaged over trial, time, and species (B).





Short title: Influence of irrigation timing

Chapter IV. Influence of post-treatment irrigation timings and herbicide placement on bermudagrass and goosegrass [*Eleusine indica* (L.) Gaertn.] response to low-rate topramezone and metribuzin programs

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Abstract

Immediate, post-treatment irrigation has been proposed as a method to reduce bermudagrass phytotoxicity from topramezone. Immediate irrigation is impractical as it does not allow time for applying herbicide. There is also insufficient evidence regarding how post-treatment irrigation, immediate or otherwise, influences mature goosegrass [*Eleusine indica* (L.) Gaertn.] control from topramezone or low-dose topramezone plus metribuzin programs. We sought to investigate bermudagrass turf and goosegrass response to immediate, 15-minute, and 30-minute post-treatment irrigation compared to no irrigation following topramezone at 12.3 g ae ha⁻¹, the lowest labeled rate, or topramezone at 6.1 g ha⁻¹ plus metribuzin at 210 g ai ha⁻¹. We also

evaluated placement of each herbicide and the combination on soil, foliage, and soil plus foliage in an attempt to elucidate the mechanisms involved in differential responses between species and herbicide mixtures. Responses were largely dependent on trial due to bermudagrass injury from high-dose topramezone being nearly eliminated by immediate irrigation in one trial and only slightly affected in another. When post-treatment irrigation was postponed for 15 or 30 minutes, topramezone alone injured bermudagrass unacceptably in both trials. Bermudagrass was injured less by low-dose topramezone plus metribuzin than by high-does topramezone and the former's impact on bermudagrass was dramatically reduced by irrigation. All post-treatment irrigation timings reduced goosegrass control compared to no post-treatment irrigation. The herbicide placement study suggested that topramezone control of goosegrass is highly dependent on foliar uptake and phytotoxicity of both bermudagrass and goosegrass is greater from topramezone than from metribuzin. Thus, post-treatment irrigation likely reduces topramezone rate load with a concomitant effect on plant phytotoxicity of both species. Metribuzin was shown to reduce 21-d cumulative clipping weight and tiller production of plants, and this may be a mechanism by which it reduces foliar white discoloration when mixed with topramezone.

Keywords:

Days over injury threshold; Digital image analysis; Late-season rescue treatments; Latitude 36; foliar or soil applied herbicide; turfgrass; white discoloration

Introduction

In recent years, goosegrass [Eleusine indica (L.) Gaertn.] control in bermudagrass [Cynodon dactylon (L.) Pers. x Cynodon transvaalensis Burtt Davy] turf has become more difficult due to the decline in effective herbicide options caused by increases in herbicide resistant populations (e.g., prodiamine and oxadiazon), loss of herbicides (e.g., diclofop), and increased use restrictions (e.g., monosodium acid methanearsonate) (Breeden et al. 2017; Keigwin 2013; McCullough 2014; McCullough et al. 2013; McElroy et al. 2017). With reduced control options, turf managers have begun to rely on more expensive herbicide options, such as foramsulfuroncontaining products, or more injurious options such as metribuzin and topramezone (Busey 2004; Brewer et al. 2016 Cox et al. 2017; Elmore et al. 2011; Johnson 1980; McCullough 2012). Foramsulfuron can effectively control less-than-three-tiller goosegrass when applied sequentially at or above 44 g ai ha⁻¹, but control declines as goosegrass matures (Busey 2004; McCullough et al. 2012). Metribuzin can more economically control mature goosegrass compared to foramsulfuron, but metribuzin rates must be greater than 210 g ai ha⁻¹ (Busey 2004; Johnson 1980; Kerr et al. 2019b). Researchers have observed that these rates of metribuzin injured bermudagrass unacceptably (Busey 2004; Johnson 1980; Kerr et al. 2019a, 2019b).

Topramezone, a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, has recently been labeled for use in bermudagrass turf for goosegrass control at rates between 12.3 and 18.4 g ae ha⁻¹ (Anonymous 2018; Senseman 2007). These rates are two to three times lower than most cool-season turfgrass rates due to bermudagrass sensitivity to topramezone (Anonymous 2018; Brewer et al. 2017; Cox et al. 2016; Kerr et al. 2019a, 2019b). In fact, bermudagrass has been injured unacceptably from topramezone at rates as low as 6.1 g ha⁻¹ (Cox et al. 2016). Multiple researchers have evaluated topramezone admixtures with fertility products and other herbicides

in an attempt to reduce the bleaching symptoms to an acceptable level. Cox et al. (2016) observed that triclopyr effectively reduced the bleaching symptoms caused by topramezone, but increased bermudagrass necrosis, stunting, and injury duration in comparison to topramezone applied alone. Boyd et al. (2020a, 2020b) observed similar results with triclopyr, and observed that chelated iron could also reduce the bermudagrass bleaching caused by topramezone.

Immediate post-treatment irrigation is another strategy that has recently been employed to reduce bermudagrass injury from multiple herbicides including metribuzin and topramezone. Kerr et al. (2019a) observed that immediate, post-treatment irrigation significantly reduced bermudagrass injury caused by metribuzin applied at 420 g ai ha⁻¹ from 35 to 6% one week after treatment, but bermudagrass injury from topramezone was not reduced by irrigation in this study. In another study, topramezone applied at 12.3 g ha⁻¹, metribuzin applied at 420 g ha⁻¹, and the combination of topramezone plus metribuzin injured bermudagrass and controlled mature goosegrass significantly less when immediate irrigation was applied (Kerr et al. 2019b). The concept of post-treatment irrigation has merit based on some examples of reduced bermudagrass discoloration and conserved control of seedling goosegrass (Kerr et al. 2019b).

Our previous research in Virginia has refined the herbicide rates in a topramezone plus metribuzin admixture to maximize bermudagrass safety while conserving mature goosegrass control and confirmed its performance at 21 Virginia locations (unpublished data). Adding post-treatment irrigation could further reduce bermudagrass injury based on earlier reports. However, only immediate irrigation has previously been tested (Kerr et al. 2019a, 2019b) and this method is not only impractical but impossible to implement using conventional turfgrass equipment and irrigation practices. According to the Golf Course Superintendents Association of America, the 18 fairways on an average golf course comprise 12 ha or 24% of the course (GCSAA 2017). By

simply dividing total fairway hectarage by 18 holes per course, an average golf fairway of 0.67 ha is equivalent to that of the typical athletic field (STMA 2018). Assuming a 0.7-ha golf fairway or athletic field, a turf sprayer with a 5.5-m wide boom traveling 6.5 kph would require at least 12 minutes to treat without turn-around time. Thus, the earliest practical irrigation timing for most situations would be 15 minutes after application or longer depending on size of the area treated. Elucidating how irrigation will affect plant response is further confounded by possible differences between topramezone and metribuzin regarding foliar versus root absorption. Neither herbicide has been evaluated for differential bermudagrass or goosegrass response based on placement to soil versus foliage.

Since immediate irrigation following topramezone yielded varying results in different trials previously (Kerr et al. 2019a, 2019b), we hypothesized that post-treatment irrigation at 15 or 30 min after treatment would not reduce bermudagrass injury equivalent to immediate irrigation. Our objectives were to determine how immediate post-treatment irrigation compares to more practical irrigation timings of 15 and 30 minutes after treatment or no post-treatment irrigation for bermudagrass and goosegrass response. We also sought to elucidate possible mechanisms that govern post-treatment irrigation impacts on bermudagrass and goosegrass response to either topramezone, metribuzin or the combination thereof when the treatments were applied to soil, foliage, or soil plus foliage.

Materials and Methods

Field Assessment of Post-Treatment Irrigation Timings

Four field trials were conducted as duplicate sites of a randomized complete block experiment with four replications per trial to assess goosegrass control and bermudagrass tolerance to herbicides followed by irrigation at varying times after treatment. The treatments were arranged

in a factorial design with two herbicides and four irrigation timings (none, immediate, 15 minutes and 30 minutes after herbicide treatment). Herbicides treatments included topramezone at 12.3 g ha⁻¹ alone (Pylex®, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709, USA) and topramezone at 6.1 g ha⁻¹ plus metribuzin at 210 g ha⁻¹ (Sencor®, Bayer Environmental Science, A Division of Bayer Crop Science LP, 5000 CentreGreen Way, Suite 400 Cary, NC 27513, USA), each applied with 0.5% v/v of methylated vegetable oil (MVO) (Dyne-Amic®, Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN 38017, USA). Herbicides were applied using a CO₂-pressurized sprayer calibrated to deliver 374 L ha⁻¹ at 289 kPa via four 11006 TTI Teejet nozzles (Teejet; Spraying Systems Co., Glendale Heights, IL 62703) which covered a 1.83 m swath. Plots received 0.25 cm of irrigation that occurred over a two-minute period. A nontreated and check was included for comparison.

The two bermudagrass tolerance trials were initiated at the Glade Road Research Facility (GRF) (37.23°N, 80.44°W) on the main campus of Virginia Tech in Blacksburg, VA, on August 16, 2018 (GRF-18), and August 26, 2019 (GRF-19), on a 'Latitude 36' bermudagrass research fairway. Both trials were maintained at 1.8 cm, and 37 kg N ha⁻¹ fertility and fungicides were applied every two to four weeks to maintain proper turf health and quality. Supplemental irrigation was supplied during both trials to prevent plant wilt. Soil was a Groseclose-Urban land complex loam (clayey, mixed, mesic Typic Hapludults) with a pH of 6.1 and 3.6% organic matter. The two goosegrass control trials were initiated at the Turfgrass Research Center (TRC) (37.22°N, 80.41°W) in Blacksburg, VA on August 16, 2018 (TRC-18), and August 28, 2019 (TRC-19), on a bermudagrass research fairway heavily infested with goosegrass. Soil was a Groseclose-Urban land complex loam (clayey, mixed, mesic Typic Hapludults) with a pH of 6.1 and 2.9% organic matter. Both trials were maintained at 3.8 cm. The goosegrass was between the

15- to 25-tiller growth stage at initiation of both trials. Plot sizes for the field sites were 1.83 m by 1.8 m in 2018 and 1.8 m by 3.6 m in 2019.

Data were assessed at 0, 0.7 1, 2, 3, 4, 6, and 8 weeks after treatment (WAT). Normalized difference vegetation index (NDVI) was collected using a multispectral analyzer (Crop CircleTM Model ACS-210, Holland Scientific Inc., 6001 South 58th Street, Lincoln, NE 68516) that scanned the center 0.45 m by 1.8 to 3.6 m of each plot and collected 40 ± 2 readings m⁻². Plot images were digitally assessed for green turf cover at 0.7, 1 and 2 WAT by Field Analyzer (Turf Analyzer; Fayetteville, AR 72701) with selected settings of low hue from 75 to 85, high hue at 360, low saturation from 25 to 29, high saturation at 100, low brightness at 0, and high brightness from 65 to 71. Before green cover was assessed, a grid was selected with an Xoffset of 20 and Y-offset of 20 to reduce any variable edge effect. Goosegrass plant counts were assessed 8 WAT by counting the total number of plants plot⁻¹ and converting to plants m⁻². Bermudagrass injury, bermudagrass foliar bleaching, and goosegrass control were visually assessed on a 0 to 100% scale (Frans et al. 1986) at all rating dates. To account for repeated measures over time, bermudagrass injury data were expressed as the maximum observed injury and white discoloration at any evaluation date and the number of days over an injury threshold of 30% (DOT₃₀). These injury DOT₃₀ values were calculated assuming linear trends in changes to bermudagrass injury between assessment dates. Bermudagrass white discoloration days over a threshold of 10% (DOT₁₀) were also calculated. Days over threshold values, as have been reported in other studies (Cox et al. 2017), reflect temporal trends in objectionable turfgrass injury that are an important component to managing turfgrass aesthetics over time. The minimum value for turf NDVI and green cover were also recorded for each experimental unit as the minimum observed value at any assessment date.

These responses were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect replicate, trial, herbicide treatment, irrigation timing, herbicide by irrigation timing, and all interactions of these effects or interactions with trial. Trial was considered a random variable in the combined analysis and mean squares of fixed effects or interactions were tested by the mean square of each effect's interaction with trial (McIntosh 1983). Appropriate means were separated with Fisher's Protected LSD test at $P \le 0.05$. If trial interactions were significant, data were presented separately by trial, otherwise, data were pooled. In the case of NDVI and turf green cover, data from the nontreated check was compared to each level of significant effects or interactions via single-degree-of-freedom tests.

Influence of Herbicide Placement on Bermudagrass and Goosegrass Response

A greenhouse experiment was initiated on October 19, 2020 (GH-1) and October 23, 2020 (GH-2) in a Quonset-style greenhouse at GRF. Both trials were established as a RCBD with four replications and a two by four by three factorial treatment design, which includes two plant species (bermudagrass and goosegrass), four herbicide treatments, and three application placements. The herbicide treatments include a nontreated check, topramezone applied at 6.1 g ha⁻¹, metribuzin applied at 210 g ha⁻¹, and topramezone plus metribuzin. All treatments were applied with MVO at 0.5% v/v. Both trials were sprayed using a CO₂-pressurized spray chamber calibrated to deliver 374 L ha⁻¹ at 289 kPa and fitted with a single Teejet 8002 even flat fan.

All herbicide treatments were applied to foliage only, soil only, and soil plus foliage.

Foliage-only treatments were administered by inserting cotton balls to completely cover exposed soil around plants in each pot. These cotton balls were removed and discarded following treatment. After two d, foliage-only-treated pots were turned on their side and the foliage was rinsed with a garden-hose-fitted spray wand for five seconds to remove any unabsorbed

herbicide from leaves without allowing exposure to underlying soil. Soil-only treatments were administered by carefully wrapping all foliage with aluminum foil and constricting the foil to minimize surface area prior to spraying pots. Soil plus foliage treatments were sprayed over the top of pots with no modifications.

The two greenhouse trials were established by removing sod from a 'Premier Pro brand' bermudagrass research fairway. All soil from the root system of the harvested sod was removed, and then 2 to 3 cm plugs were planted into 10.2-cm pots in a soil and sand mix (2:1 by wt). The soil used was a Groseclose-Urban land complex loam (clayey, mixed, mesic Typic Hapludults) with a pH of 6.0 and 3.1% organic matter. Goosegrass seed were sown into a flat containing sand. When seedlings reached the two-to-three-leaf stage, they were transplanted into pots as described for bermudagrass. These plants were allowed to mature to the 4- to 7-tiller stage before herbicides were applied. All plants were fertilized 25 kg N ha⁻¹ once per week to maintain proper plant growth. Automated irrigation was supplied daily to prevent plant wilt. The greenhouse had supplemental lighting that was set at a 14-hr daylength. The first study used sodium halide lights with 650 µmol m⁻² sec⁻¹ output of photosynthetically active radiation while the second study used mercury vapor lamps with 430 µmol m⁻² sec⁻¹ output of photosynthetically active radiation. The greenhouse was maintained at 26/24 °C day/night temperature.

Data were collected at 0, 3, 5, 7, 10, 14, and 21 days after treatment (DAT) which included visually-assessed bermudagrass injury/bleaching and goosegrass control/bleaching, goosegrass tiller counts, and normalized difference vegetation index (NDVI). All visual data were assessed similar to the field trials. Normalized difference vegetative index was assessed regularly as a measure of plant health using a Spectral Evolution PSR-1100 hyperspectral radiometer (Spectral Evolution; 26 Parkridge Rd. Suite 104, Haverhill, MA 01835, USA) with

leaf-clip attachment. Bermudagrass and goosegrass were mown every three days with clippings collected to evaluate differences in cumulative clipping yield. Final foliar biomass for both species was also collected at the conclusion of each study. The clippings and final biomass were dried at 22°C for 72 hours in a chromatography oven and weighed. Data were subjected to ANOVA with sums of squares partitioned to reflect the two by four by three factorial treatment design and trial effects as previously described. Mean separation procedures were the same as in the field study.

Results and Discussion

Field Assessment of Post-Treatment Irrigation Timings

The trial-by-irrigation-by-herbicide interaction was significant ($P \le 0.0138$) for bermudagrass injury maxima, bermudagrass injury DOT₃₀, white discoloration maxima, white discoloration DOT₁₀, and bermudagrass green cover minima (Table 1). This interaction was mainly caused by variable response of bermudagrass to topramezone alone at the immediate and 15-minute-after-treatment irrigation timings. In the first trial, irrigation had generally less effect on bermudagrass response to topramezone than in the second trial. We feel that inconsistencies in our watering techniques between the two trials may be to blame. In the first trial, patterns of white discoloration were apparent and indicative of nonuniform irrigation distribution. In the second trial, the hose wand was waved back and forth and side to side compared to a unidirectional pattern in the first trial. There have only been two studies that evaluated immediate, post-treatment irrigation following topramezone application to bermudagrass turf. Immediate, post-treatment irrigation did not influence bermudagrass injury in one study (Kerr et al. 2019a), and reduced bermudagrass injury from 53% without irrigation to 11% with irrigation in another study (Kerr et al. 2019b). The inconsistencies between previous reports and in the current study between trials, brings reliability of post-treatment irrigation for improving

bermudagrass tolerance to topramezone into question. Despite the inconsistencies between trials for immediate and 15-minute-post-treatment irrigation, other irrigation treatments and trends in bermudagrass response between topramezone alone and topramezone plus metribuzin were quite consistent (Table 1).

Maximum bermudagrass injury following low-dose topramezone plus metribuzin was always less than that of high-dose topramezone alone, except for topramezone followed by immediate irrigation in trial 2 (Table 1). At this time, research has not been published that evaluates topramezone at the low-dose of 6.1 g ha⁻¹ plus metribuzin at 210 g ai⁻¹. When a similar admixture was applied at twice the rate of each herbicide, bermudagrass was injured 82% and more than topramezone alone (Kerr et al. 2019b). Lindsey et al. (2019, 2020) observed that topramezone applied at 10 g ha⁻¹ plus metribuzin applied at 90 or 100 g ha⁻¹ injured bermudagrass 85 to 78%, which is more than maximum injury by topramezone plus metribuzin with no irrigation in our study. Kerr et al. (2019b) and Cox et al. (2016) observed substantial injury following treatment of topramezone at 12.3 g ha⁻¹ to bermudagrass turf without irrigation, similar to the current study.

The 15-minute and 30-minute irrigation intervals for topramezone did not reduce maximum injury in trial 1 and slightly reduced maximum injury in trial 2 (Table 1). These later irrigation timings did not reduce bermudagrass injury nearly as much as immediate irrigation, thus supporting our hypothesis. We did not expect, however, that the more practical 15- and 30-minute post-treatment irrigation timings would reduce maximum bermudagrass injury by low-dose topramezone plus metribuzin as was observed (Table 1). For low-dose topramezone plus metribuzin, immediate, 15-minute, and 30-minute irrigation intervals reduced bermudagrass injury near or below the 30% acceptable threshold in all but one case in trial 1 (Table 1).

Bermudagrass injury DOT₃₀ was reduced much more by low-dose topramezone plus metribuzin than by any post-treatment irrigation practice save that of immediate irrigation in trial 2 (Table 1). Topramezone at 12.3 g ha⁻¹ alone caused above-threshold injury levels for at least 10 days regardless of irrigation program with the exception of immediate irrigation in trial 2. Similar to the current studies, Cox et al. (2016) observed that topramezone applied once at 6.1 or 12.3 g ha⁻¹ caused 11 to 16 days over a 30% injury threshold. In contrast to high-dose topramezone alone, low-dose topramezone plus metribuzin combined with any post-treatment irrigation timing reduced bermudagrass DOT₃₀ to not more than 4.8 days. The reduced DOT₃₀ when post-treatment irrigation was added following topramezone plus metribuzin is encouraging, assuming these programs will still control goosegrass.

The white discoloration maxima followed similar trends as bermudagrass injury maxima albeit with greater reductions caused by adding metribuzin to low-dose topramezone (Table 1). Post-treatment irrigation only reduced bermudagrass white discoloration to below 10% in one of six cases for high-dose topramezone alone and five of six cases for low-dose topramezone plus metribuzin. These white discoloration maxima values trend well with white discoloration DOT₁₀. The more practical irrigation times of 15 and 30 minutes after topramezone treatment still caused bermudagrass white discoloration above 10% for 12 to 15 days compared to 0 to 1.2 days when these later irrigation timings were applied following low-dose topramezone plus metribuzin. The white discoloration DOT₁₀ caused by topramezone in these trials is similar to observations made by Cox et al. (2016).

The interaction of irrigation timing and herbicide was significant (P = 0.0439) for bermudagrass turf NDVI minima, and not dependent on trial (P > 0.05). Thus, data were pooled over the two trial sites for presentation in Table 1. The nontreated turf had a minimum NDVI of

0.7306 and this was equivalent to high-dose topramezone followed by immediate irrigation and low-dose topramezone plus metribuzin followed by any timing of irrigation. Non-irrigated turf following any herbicide and 15 or 30-minute post-treatment irrigation following high-dose topramezone had significantly lower NDVI compared to the nontreated check (Table 1). These trends in NDVI align well with other bermudagrass response variables, albeit with smaller magnitude between treatment effects. This smaller magnitude of differences compared to visual ratings suggest that the multispectral analyzer either detects green turf that may reside underneath white foliage or detects higher infrared reflection, which is normalized in the equation. Otherwise, it is hard to reconcile such high levels of visual phytotoxicity yielding such small differences in NDVI. In research to evaluate aerial versus ground-based NDVI assessments compared to visually estimated turf chlorosis, examples where turf chlorosis was similar between herbicides of differing modes of action led to widely variable NDVI values, suggesting that NDVI may not correctly estimate turfgrass health equivalently across all types of stress (Zhang et al. 2019).

Digitally assessed bermudagrass green cover minima mirrored trends in bermudagrass injury maxima (Table 1). These data speak to the accuracy of visually estimated data in these studies as both injury maxima and green cover minima are based on multiple assessments made over 8 weeks and represent a near perfect negative correlation based on regression of means in (Table 1). Green cover was conserved compared to nontreated turf in only one instance where immediate irrigation followed high-dose topramezone in trial 2 and in all instances where immediate or 15-minute, post-treatment irrigation followed low-dose topramezone plus metribuzin.

The interaction of irrigation timing by herbicide was significant for goosegrass control 8 WAT (P = 0.0404) and not dependent on trial (P > 0.05). When post-treatment irrigation was not applied, high-dose topramezone and low-dose topramezone plus metribuzin controlled goosegrass greater than 90% and equivalently (Table 2). Unfortunately, a substantial decrease in goosegrass control resulted from all post-treatment irrigation timings (Table 2) and this effect worsened with irrigation timings that were more effective at reducing bermudagrass injury responses (Table 1). For example, goosegrass control was reduced 16 and 51% when immediate, post-treatment irrigation was applied following high-dose topramezone or low-dose topramezone plus metribuzin, respectively. The interaction of trial by herbicide was significant (P = 0.0222) for final goosegrass plant counts. When averaged over all irrigation levels, the herbicides resulted in equivalent goosegrass plant densities in trial 1 and lower plant densities by topramezone alone in trial 2.

Influence of Herbicide Placement on Bermudagrass and Goosegrass Response

The interaction of plant species by herbicide by placement was significant (P = < 0.05) for plant injury maxima and plant NDVI minima and no effect was dependent on trial (P > 0.05). Bermudagrass was injured 37 to 47% and more than other treatments when either high-dose topramezone or low-dose topramezone plus metribuzin were applied to soil plus foliage (Table 3). Foliar-only application of these two herbicides was considerably more injurious than soil application and trailed the more injurious soil plus foliar applications by about 10%. Metribuzin did not injure bermudagrass more than 17% and soil-only applications of any herbicide did not injure bermudagrass more than 3.9%. Similar trends were noted for goosegrass injury in that foliar-only or soil-plus-foliar applications injured goosegrass 96 to 100%. Soil-only applications of topramezone injured goosegrass not more than 3.5% and this injury rose to 23% when

metribuzin was added (Table 3). These data suggest that topramezone is responsible for most of the plant response from the topramezone plus metribuzin combination and that topramezone is highly dependent on foliar absorption. The soil-only contribution of metribuzin, however, is not insubstantial. Plant NDVI minima support plant injury data in that foliar and soil-plus-foliar applications to either plant species significantly reduced NDVI compared to nontreated plants while soil-only applications to bermudagrass did not influence NDVI (Table 3). Goosegrass NDVI values also generally mirrored trends in goosegrass injury.

The interaction of plant species by herbicide by placement was significant (P < 0.05) for percentage of nontreated plant biomass, percentage of nontreated 21-d cumulative clipping dry weight, and post treatment goosegrass tiller growth and these effects were not dependent on trial. Final foliar biomass was equivalent for all herbicides within each application placement (Table 6). Goosegrass biomass was reduced to no more than 13% of the nontreated plants when topramezone-containing treatments were applied to foliage or foliage plus soil. Soil application of metribuzin reduced goosegrass biomass about 50%. These data suggest that the topramezone plus metribuzin combination effects goosegrass control by alternate routes of uptake depending on herbicide. Topramezone appears more dependent on foliar uptake while metribuzin is more active via uptake from soil.

Bermudagrass cumulative clipping dry weight was 85% of that produced by nontreated plants when foliar-only treated with topramezone only and 61 to 64% of the nontreated when treated with metribuzin (Table 4). A similar trend was evident when these herbicides were applied to soil plus foliage. This observation may partially explain how metribuzin reduces white discoloration when included as an admixture with topramezone. Goddard et al. (2010) showed that white-discolored leaves of tall fescue and several weeds were limited to new leaves

that were produced after mesotrione, a related HPPD-inhibiting herbicide, was applied. If metribuzin causes a transient reduction in leaf growth, as the 21-d cumulative clipping weights suggest (Table 4), then white discoloration would be reduced since new leaves aren't growing. The trends in 21-d cumulative clipping weights of goosegrass exhibit similar trends to that of foliar biomass with respect to herbicide and placement (Table 4).

In the 21 days following treatment, nontreated plants added 14 to 16 additional tillers and this was equivalent to topramezone applied only to soil. The addition of metribuzin reduces tiller count when added to topramezone (Table 4) and this trend further supports the reductions in cumulative clipping weights caused by metribuzin (Table 4). Following topramezone treatment, goosegrass continues to grow for the first few weeks, and adding metribuzin helps to reduce this growth. This impact of metribuzin may partially contribute to goosegrass control in field settings simply by assisting in competitive displacement of goosegrass by bermudagrass turf since goosegrass is not growing.

Results of these studies suggest that post-treatment irrigation likely reduces bermudagrass response by rinsing topramezone from plant leaves and effectively reducing the herbicide rate. Inconsistencies in goosegrass control may occur if irrigation occurs rapidly enough to prevent a lethal dose being absorbed into the foliage. Topramezone absorbs into goosegrass more rapidly than bermudagrass and metribuzin decreases absorption rate by both species (unpublished data). The reduced absorption caused by metribuzin admixture (unpublished data) and dependence on foliar uptake by topramezone to reduce goosegrass biomass (Table 4) explains why goosegrass control was more severely reduced when post-treatment irrigation followed the mixture (Table 2). Metribuzin reduces leaf and tiller production of affected species in the first 21 d after

treatment and likely contributes to the substantial decrease in white foliar discoloration when admixtures of metribuzin are added to topramezone compared to topramezone alone.

The dramatic improvement in bermudagrass safety when 15-minute, post-treatment irrigation followed low-dose topramezone plus metribuzin and concomitant 70% mature goosegrass control from this treatment is encouraging. The irrigation reduced goosegrass control from 91 to 70% but this limitation could possibly be overcome by including a sequential treatment of the program at a 3-wk interval. Other studies have shown that sequential topramezone treatments can improve weed control compared to single treatments (Cox et al. 2017; Elmore et al. 2011, Kerr et al. 2019b). We find immediate irrigation too impactful on goosegrass control and impossible to implement using current sprayer and irrigation technology in the turfgrass industry. One limitation of this, and other studies (Kerr et al. 2019a, 2019b) is that irrigation applied to small plots with a hose-end wand mimics syringing rather than actual turfgrass irrigation systems. Future work should evaluate topramezone plus metribuzin programs on in-use turfgrass facilities to determine if practical, post-treatment irrigation regimes will respond similarly to the current study and if such treatment plus irrigation programs can control mature goosegrass completely with sequential treatments.

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Table 1. Influence of herbicide treatments and irrigation intervals on bermudagrass injury and white discoloration maxima, days over a 30% bermudagrass injury threshold (DOT₃₀) and a 10% bermudagrass white discoloration threshold (DOT₁₀), minimum turf normalized difference vegetation index (NDVI) and minimum bermudagrass green cover.

	Bermudagrass injury maxima				Bermudagrass injury DOT ₃₀				
Irrigation interval	Topran	nezone ^a	Topram ^b -	+ metribuzin	Topran	nezone	Topram + n	netribuzin	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	
_					no. of days				
None	97*	92*	74*	59*	15*	18*	9.5*	9.1*	
Immediate	70*	10*	23*	28*	10*	0	0.7*	0.7	
15 min	93*	69*	30*	21*	12*	13*	1.6*	0*	
30 min	88*	73*	45*	31*	12*	13*	4.8*	1.2*	
LSD (0.05)	9.7	3.4	14	8	2.2	1.9	3.7	1.5	
	Bermudagrass white discoloration maxima				Bermu	dagrass white	discoloration	DOT ₁₀	
	Topramezone Topram + metribuzin		Topra	amezone	Topram + metribuzin				
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	
			%		no. of days				
None	96*	88*	49*	34*	15*	19*	11*	10*	
Immediate	59*	0.8*	2*	8.8*	12*	0	0*	0	
15 min	90*	48*	5.5*	4.5*	12*	13*	0*	0*	
30 min	85*	55*	9.3*	8.8*	13*	15*	1.2*	0*	
LSD (0.05)	15	6.2	5.4	8.3	1.0	1.9	NS	1.0	
<u>-</u>					Berm	udagrass greei	n cover DIA n	ninima	
_		Bermudagra	ss NDVI mii	nima	Topr	amezone	Topram + metribuzin		
	Top	ramezone	Topram + metribuzin		Trial 1	Trial 2	Trial 1	Trial 2	
		i	ndex						
None	0.	6061*+		0.6694*+	2*+		37*+	39*+	
Immediate	(0.7036		0.7285	47*+	83	80*	75	
15 min	0.	6671*+		0.7206*		27*+	68*	78*	
30 min	0.	6478*+		0.6970	7.9*+	30+	63*+	57+	

LSD (0.05)	0.0524	0.0489	12	20	20	27
Nontreated check	0.7306		84	87	84	87

^{*} Herbicide difference; + Different from nontreated check.

^aTopramezone alone applied at 12.3 g ae ha⁻¹; Topramezone + metribuzin applied at 6.13 g ha⁻¹ + 210 g ai ha⁻¹.

^bAbbreviations: Topram, Topramezone.

Table 2. Influence of herbicide, irrigation, and trial on goosegrass [*Eleusine indica* (L.) Gaertn.] control and goosegrass plant counts m⁻².

	Goosegrass control					
Irrigation Interval	Topramezone ^a	Topram ^b + metribuzin				
None	97	91				
Immediate	81*	40*				
15 min	88*	70*				
30 min	89*	71*				
LSD (0.05)	7	15				
	Goosegrass plant density					
		— no. m ⁻² ———				
Trial 1	10	19				
Trial 2	28*	70*				

 $^{^{}a}$ Topramezone alone applied at 12.3 g ae ha $^{-1}$; Topramezone + metribuzin applied at 6.13 g ha $^{-1}$ + 210 g ai ha $^{-1}$.

^bAbbreviations: Topram, Topramezone.

Table 3. Influence of herbicide and placement on bermudagrass and goosegrass [*Eleusine indica* (L.) Gaertn.] injury maxima and normalized difference vegetation index (NDVI) minima.

		Plant injury maxima								
			Bermudagrass		-		Goosegrass			
				Foliage +	_			Foliage +	-	
Herbicide	Rate ^a	Foliage	Soil	Soil	LSD (0.05)	Foliage	Soil	Soil	LSD (0.05)	
	g ha ⁻¹		%				%			
Topramezone	6.1	28	1.8	37	8.2	96	3.5	97	5.1	
Metribuzin	210	13	3.9	17	6.1	27	25	33	NS	
Topram + metrib ^b	6.1 + 210	31	3.9	47	7.2	99	23	100	13	
LSD (0.05)		8.8	NS	7.9		5.3	17	5.2		
			Plant NDVI minima							
Topramezone	6.1	0.532	0.584	0.449	0.08	0.011	0.683	0.011	0.033	
Metribuzin	210	0.475	0.562	0.526	NS	0.446	0.591	0.574	0.071	
Topram + metrib	6.1 + 210	0.533	0.582	0.426	0.094	0.016	0.539	0.033	0.092	
No herbicide		0.646	0.634	0.592	NS	0.596	0.605	0.676	0.063	
LSD (0.05)		0.096	NS	0.069		0.067	0.076	0.059		

^aRates given as acid equivalency for topramezone and active ingredient for metribuzin.

^bAbbreviations: Topram, Topramezone; metrib, metribuzin.

Table 4. Influence of herbicide and placement on bermudagrass and goosegrass [*Eleusine indica* (L.) Gaertn.] final foliar biomass, cumulative clipping weight, and post-treatment tiller growth.

		Final foliar biomass (% of check)							
	- -	Bermudagrass				Goosegrass			_
	-			Foliage +	LSD			Foliage +	LSD
Herbicide	Rate ^b	Foliage	Soil	Soil	(0.05)	Foliage	Soil	Soil	(0.05)
	g ha ⁻¹		%				%		
Topramezone	6.1	85	110	92	NS	13	110	12	21
Metribuzin	210	70	102	80	28	74	53	51	22
Topram + metrib ^a	6.1 + 210	71	99	74	26	5.1	57	3	17
LSD (0.05)		NS	NS	NS		14	29	11	
		21-d cumulative clipping weight (% of check)							
			%				%		_
Topramezone	6.1	85	98	94	NS	19	94	16	12
Metribuzin	210	64	91	66	21	44	47	34	NS
Topram + metrib	6.1 + 210	61	103	51	18	5.1	49	5.8	20
LSD (0.05)		23	NS	21		9.3	26	6.8	
		Post-treatment tiller growth (no. plant ⁻¹)							
		tiller no. — tiller no. — tiller no. —					_		
Topramezone	6.1					3.8	17	4.3	2.9
Metribuzin	210					13	8.5	12	NS
Topram + metrib	6.1 + 210					0.8	6.4	2	4.4
NTC ^a						14	16	16	NS
LSD (0.05)						3.7	4.9	4.3	

^aAbbreviations: Topram, topramezone; metrib, metribuzin; NTC, nontreated check.

^bRates given as acid equivalency for topramezone and active ingredient for metribuzin.

Short title: Grass weed control on greens

Chapter V. Investigating low-dose herbicide programs for goosegrass (*Eleusine indica*) and smooth crabgrass (*Digitaria ischaemum*) control on creeping bentgrass greens

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Abstract

Of the four registered herbicides for smooth crabgrass or goosegrass control on creeping bentgrass golf greens, none control weedy grasses for the entire season or control weeds postemergence when applied once at labeled rates, and three prohibit use repeatedly or during stressful summer conditions. We hypothesized that frequent application of herbicides applied at low doses could provide season-long summer grass control while minimizing turf injury. Seven field experiments were conducted on creeping bentgrass greens to evaluate various herbicides applied monthly, biweekly, or weekly for postemergence and residual control of goosegrass and smooth crabgrass as well as creeping bentgrass putting green tolerance. Metamifop applied twice monthly at 200 g ai ha⁻¹, topramezone applied eight times weekly at 1.5 g ae ha⁻¹, and siduron applied weekly at 5.6 kg ai ha⁻¹ or four times biweekly at 11 kg ha⁻¹ did not injure creeping bentgrass greater than 10% and maintained creeping bentgrass quality and cover equivalent to

nontreated turf. Weekly or biweekly programs of fenoxaprop or quinclorac caused unacceptable injury and quality decline. Metamifop applied monthly and either fenoxaprop program controlled both smooth crabgrass and goosegrass 97 to 99% throughout the growing season. Programs containing either quinclorac or siduron controlled smooth crabgrass 99 to 100% but did not control goosegrass greater than 39%. Topramezone applied weekly, biweekly, or monthly at a total seasonal rate load of 12 g ha⁻¹ controlled smooth crabgrass 69 to 77% and goosegrass 93 to 98%. In additional studies, siduron applied five times biweekly did not injure creeping bentgrass greens and controlled smooth crabgrass acceptably at seasonal, cumulative rates between 17 and 65 kg ai ha⁻¹. This method of frequent, low-dose herbicide treatment to control smooth crabgrass and goosegrass on golf greens is novel and could be legally implemented currently with siduron.

Nomenclature:

fenoxaprop; metamifop; quinclorac; siduron; topramezone; creeping bentgrass, *Agrostis* stolonifera L.; goosegrass, *Eleusine indica* (L.) Gaertn.; smooth crabgrass, *Digitaria ischaemum* (Schreb.) Schreb. ex. Muhl.

Keywords:

ACCase inhibitor; digital image analysis; field analyzer software; HPPD inhibitor; photosystem II inhibitor; turfgrass injury and quality

Introduction

There are four herbicides labeled for preemergence control of goosegrass [Eleusine indica (L.) Gaertn.] and smooth crabgrass [Digitaria ischaemum (Schreb.) Schreb. Ex Muhl.] on creeping bentgrass (Agrostis stolonifera L.) golf greens. These include bensulide, dithiopyr, oxadiazon, and siduron (Anonymous 2014, 2016, 2017, 2018a; Callahan 1986; Hart et al. 2004; Patton and Weisenberger 2017). At the rates used on golf greens, these herbicides rarely provided residual protection to prevent summer annual grass emergence for more than a few months (Callahan 1986; Dernoeden et al. 1984; Johnson 1994b). Since goosegrass and smooth crabgrass emergence patterns (Chauhan and Johnson 2008; Fidanza et al. 1996) overlap with creeping bentgrass summer stress, often caused by soil-borne pathogens, insects, heat, and drought (Beard 2002; Dernoeden 2000; Miller and Brotherton 2020), reapplications of these herbicides may be necessary to maintain season-long control (Chauhan and Johnson 2008; Fidanza et al. 1996; Kerr et al. 2018). In the transition zone and areas further south, goosegrass and smooth crabgrass readily infest areas on greens where the turf canopy has been compromised due to stress (Miller and Brotherton 2020; Samaranayake et al. 2008). These weed infestations are often targeted with hand removal or cutting but sometimes overwhelm available resources for their control. Of the aforementioned preemergence herbicides, only siduron is consistently safe enough to apply frequently throughout the summer, while the others have been associated with turf and root loss during hot and stressful periods (Callahan 1972; Dernoeden et al. 1993; Hart et al. 2004), and they carry labeling restrictions that would prevent their use at frequent intervals or during stressful summer conditions. Siduron has been historically marketed for use at turfgrass establishment (Hart et al. 2004; Kaminski et al. 2004; Willis et al. 2006), while programs to extend the product's limited soil residual performance to affect season-long summer grass control on golf greens have not been evaluated.

Alternate options to deal with summer annual grassy weed infestations on greens could rely on postemergence herbicides. None are currently registered for use on creeping bentgrass greens but a few have shown promise for grassy weed control in other areas or have been assessed for turf tolerance on greens in a few published studies (Carroll et al. 1992; Cooper et al. 2017; Parker et al. 2015). In creeping bentgrass mown above 0.6 cm, fenoxaprop, topramezone, and quinclorac are registered to control goosegrass, smooth crabgrass, or both weeds depending on product (Anonymous 2013, 2018b, 2019). Henry and Hart (2004) observed fenoxaprop at 40 g ai ha⁻¹ injured and reduced turf quality of 'Penn A-4' creeping bentgrass greens to an unacceptable level. Carroll et al. (1992) observed fenoxaprop applied multiple times at 27 to 45 g ha⁻¹ caused significant discoloration to creeping bentgrass. Quinclorac at 600 g ae ha⁻¹ also significantly injured green-height creeping bentgrass (Johnson 1994a). Thus, successful implementation of fenoxaprop or quinclorac on creeping bentgrass golf greens may require frequent, low-dose applications to ensure turf safety, as was limitedly evaluated for fenoxaprop by Parker et al. (2015). Topramezone has not been evaluated on golf greens but can control both goosegrass and smooth crabgrass from 6.1 to 24 g ae ha⁻¹ in other turf species (Cox et al. 2017; Brosnan et al. 2014). Metamifop is an experimental herbicide that, when using single, high-rate applications, has been safely used on creeping bentgrass greens (Parker et al. 2015) and has controlled both goosegrass (McCullough et al. 2016; Parker et al. 2015) and smooth crabgrass (Cox and Askew 2014; Parker et al. 2015).

We sought to evaluate fenoxaprop, quinclorac, siduron, and topramezone in frequent, low-dose application programs compared to metamifop and a higher rate of topramezone applied twice at monthly intervals for grassy weed control based on their performance in previously reported literature. We hypothesized that the frequent, low-dose approach would maintain

acceptable weed control while imparting turf safety to creeping bentgrass greens or extending residual performance. Therefore, field experiments were conducted to (1) evaluate creeping bentgrass, smooth crabgrass, and goosegrass response to these herbicides on golf greens and (2) determine a minimum, and more economical, siduron rate for frequent, low-dose, seasonal programs to control smooth crabgrass on creeping bentgrass golf greens.

Materials and Methods

Creeping Bentgrass and Weed Response to Low-dose Herbicide Programs

A total of five field trials were conducted on creeping bentgrass greens as randomized complete block designs with 11 treatments and three replications. Three trials were initiated on June 6, 2016, June 6, 2016, and June 1, 2017 at the Virginia Tech Turfgrass Research Center (TRC) (37.22°N, 80.41°W) in Blacksburg, VA on 'L-93' creeping bentgrass infested with smooth crabgrass (Site 1) or on adjacent areas of a fallow, weedy area of the same greens complex in 2016 (Site 2) and 2017 (Site 3). Two additional trials were initiated on June 6, 2016 and June 1, 2017 at the Glade Road Research Facility (GRF) (37.23°N, 80.44°W) in Blacksburg, VA on weed-free areas of '007' (Site 4) and 'Tyee' (Site 5) creeping bentgrass. All five trials were conducted on research greens that were built to United States Golf Association specifications (USGA 2015) and maintained at 0.32-cm moving heights for three sites grassed with creeping bentgrass, and 2.5-cm mowing height for two weedy, fallow sites. All sites were irrigated as needed to prevent wilt of weeds and the desired turfgrass. Fertility and plant protectant programs were managed similarly to in-play golf greens consisting of 4.9 kg N ha⁻¹ to maintain both healthy turfgrass and weeds. Mean and standard error of one-to-three leaf smooth crabgrass percentage cover at initiation were 10.5±1.2 at site 1, while germination at site 2 and 3 had not

occurred by trial initiation. Goosegrass had also not germinated at the time of trial initiation for site 2 and 3.

Herbicide rates, application frequencies, adjuvants, and manufacturer information are shown in Table 1. Metamifop and topramezone at 0.0061 kg ha⁻¹ were applied twice monthly, while fenoxaprop, quinclorac, siduron, and topramezone were all applied four times every two weeks (biweekly program) at a higher rate or applied eight times every week (weekly program) at a lower rate. All treatments were applied to 0.9-m-by-1.8-m plots using a CO₂-pressurized hooded sprayer calibrated to deliver 280 L ha⁻¹ at 289 kPa via two Teejet XR6502VS flat fan nozzles (Teejet Spraying Systems Co., Glendale Heights, IL 62703).

Assessments were made at 0, 1, 3, 5, 7, and/or 9 weeks after the initial treatment (WAIT) for creeping bentgrass coverage, injury, and quality at three sites, goosegrass cover, control, and shoot density at two sites, and smooth crabgrass cover, control, and shoot density at three sites. At five and nine weeks after the initial application, green cover of creeping bentgrass was assessed via digital image analysis via Field Analyzer (Turf Analyzer; Fayetteville, AR 72701) with selected settings of low hue from 101 to 107, high hue at 360, low saturation from 11 to 18, high saturation at 100, low brightness from 29 to 31, and high brightness at 100. Grid settings were selected with an X-offset of 20 and Y-offset of 20 to reduce any variable edge effect. Green cover of goosegrass and smooth crabgrass 9 WAIT were based on line intersect counts using a 0.91-m-by-0.91-m grid that contained 240 intersects at 5.72-cm increments. Final shoot density of goosegrass and smooth crabgrass was based on shoot counts plot and converted to shoots m². All other assessments of creeping bentgrass injury, coverage and smooth crabgrass and goosegrass cover and control were assessed visually and rated on a 0 to 100% scale where 0= no injury, coverage etc. and 100= complete coverage, plant death etc. (Frans et al. 1986). Creeping

bentgrass quality was visually assessed on a 1-9 scale where 1= minimum turfgrass quality, 6 is minimally acceptable turfgrass quality, and 9= maximum turfgrass quality (Krans and Morris 2007).

Turfgrass injury and quality estimates over time were converted to area under the progress curve (AUPC) using

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

where ∂ is the AUPC, i is the ordered sampling date, ni is the number of sampling dates, y is turf injury or quality measurements at a given date, and t is the time in days. The AUPC was then converted to the average per day by simply dividing the total AUPC by the total number of days in the study similar to Brewer et al. (2016). Unlike single-date analyses, this method offers a better comparison of severity and duration of specific response variables over time, and can be useful in situations when turfgrass response is assessed by repeated measures over long study durations. To determine the most severe creeping bentgrass injury caused by each treatment, maximum observed turfgrass injury and the minimum observed turfgrass quality for each experimental unit was used.

Data were subjected to analysis of variance with mean squares separated to assess replicate, trial, treatment, and trial by treatment effects. The mean square of treatment effects was tested by the mean square associated with trial by treatment (McIntosh 1983). Appropriate means were separated with Fisher's Protected LSD Test at P < 0.05.

Siduron Rate Response Experiment

Two field trials were established on June 1, 2017 and June 2, 2019 to compare siduron rates for season-long smooth crabgrass control and creeping bentgrass response. Both trials were conducted at the TRC on the same 'L-93' creeping bentgrass green as Site 1 from the previous field trials. The treatments include siduron at 3.4, 6.7, 10, and 14 kg ai ha⁻¹. Each rate was applied biweekly for a total of five applications over 10 weeks. Mean and standard error of three-leaf to two-tiller smooth crabgrass percentage cover at initiation were 8.2±1.1 for trial 1 and 16.3±2.1 for trial 2. The trial was arranged as a randomized complete block design with four replications and a plot size of 1.8 m by 1.8 m. Treatments were sprayed with a hand-held CO₂-pressurized boom calibrated to deliver 280 L ha⁻¹ at 289 kPa and equipped with Teejet TTI 11004 nozzles.

Final smooth crabgrass and creeping bentgrass coverage was assessed at 14 WAIT using line intersect counts as described previously. Creeping bentgrass injury and quality were visually assessed at 0, 1, 2, 4, 6, 8, 12, and 14 WAIT as described previously. Creeping bentgrass quality was converted to AUPC d^{-1} as described previously. Smooth crabgrass density was assessed 14 WAIT as plants plot⁻¹ and converted to plants m⁻². Data were subjected to ANOVA with sums of squares partitioned to reflect rep, trial, treatment and trial by treatment effects. Mean squares were tested to account for trial effects as previously described and means were separated using Fisher's Protected LSD test at P < 0.05 applied first to any significant trial interactions or to other effects if trial interactions were not significant.

Results and Discussion

Creeping Bentgrass Response to Low-dose Herbicide Programs

The treatment main effect was significant for creeping bentgrass maximum injury, injury AUPC d^{-1} , minimum turf quality, turf quality AUPC d^{-1} , and final turf cover at 9 WAIT (P < 0.0001, P < 0.0001, P = 0.0003, P = 0.0010, and P < 0.0001, respectively) (Table 2). There was no significant trial interaction for any of the five response variables (P \geq 0.2111), which allowed data to be averaged over three site years (Table 2).

Metamifop, both siduron application programs, and topramezone applied weekly or biweekly did not injure creeping bentgrass greater than 15% and this was less than maximum injury by both fenoxaprop rates, both quinclorac rates, and topramezone applied monthly, which was 38 to 74% (Table 2). Both Parker et al. (2015) and Cooper et al. (2017) similarly observed that multiple applications of metamifop at 0.2 kg ai ha⁻¹ injured creeping bentgrass less than 10%. Only one study has investigated repeated applications of siduron on creeping bentgrass greens. Four monthly treatments of 54 kg ha⁻¹ siduron injured greens-height creeping bentgrass 0 to 36% depending on study site (Johnson and Carrow 1993). Research on seedling creeping bentgrass or field-collected plugs suggests that variability in creeping bentgrass response to siduron could be related to cultivar (Reicher et al. 2002; Splittstoesser and Hopen 1967). Our study evaluated 'L93', '007', and 'Tyee' creeping bentgrass and none were injured by the novel frequent, low-dose siduron programs evaluated (Table 2). The creeping bentgrass cultivar 'L-93' is one of three cultivars that we utilized for our tolerance studies, and it has been observed to be a relatively tolerant cultivar (Hart et al. 2004; Reicher et al. 2002). Our data suggest that frequent, low-dose treatments of siduron reduce injury potential compared to single, high-dose treatments.

At this time, there is no published research that has evaluated greens-height (less than 1 cm) creeping bentgrass response to topramezone or bentgrass response to topramezone applied at rates less than 0.008 kg ha⁻¹. Elmore et al. (2015) observed that topramezone applied at 0.008 kg

ha⁻¹ injured single-plant creeping bentgrass seedlings 22%, which is slightly higher than the maximum injury observed in our studies by topramezone applied biweekly to mature creeping bentgrass greens. Topramezone can cause unacceptable injury to creeping bentgrass if rates are applied at 0.018 kg ha⁻¹ or greater (Brewer et al. 2016).

Turf injury AUPC d⁻¹ exhibited similar trends to that of maximum turf injury (Table 2). Fenoxaprop and quinclorac were the most injurious herbicides and deemed unacceptable for creeping bentgrass greens if applied weekly or biweekly as in these studies. Fenoxaprop applied weekly, for example, injured creeping bentgrass an average of 43% d⁻¹ based on AUPC (Table 2). This level of injury is just above the acceptable threshold of 30% and could go unnoticed on golf greens that receive routine colorant applications to improve green color. Our weekly application program for fenoxaprop injured creeping bentgrass significantly higher than observed in similar studies conducted by Parker et al. (2015) and Henry and Hart (2004). During their studies, fenoxaprop applied at similar rates to the current study injured greens-height creeping bentgrass less than 20%. We attribute this lower creeping bentgrass injury to a 3-wk fenoxaprop treatment interval in their studies (Henry and Hart 2004; Parker et al. 2015) compared to weekly treatments in our study (Table 2). Only Parker et al. (2015) reported if the less-frequent fenoxaprop treatment interval used in their studies could control smooth crabgrass at similar rates as the current studies, while their goosegrass evaluation occurred with rates 6 times higher than our studies and one or two applications. Only one peer-reviewed paper has discussed creeping bentgrass greens response to quinclorac and the herbicide was applied once at three times the highest rate used in our study (Johnson 1994a). Johnson (1994a) observed that quinclorac applied at 0.6 kg ha⁻¹ once reduced greens-height creeping bentgrass turf quality by a maximum of 42 and 51%, which is similar to the turf injury AUPC d⁻¹ caused by both quinclorac frequent, low-dose programs in our studies.

Turf quality minima and average daily turf quality based on AUPC mirrored the trends in turfgrass injury, suggesting that quality was primarily influenced by herbicide injury in these studies (Table 2). Only turf treated with metamifop, both siduron programs, and weekly topramezone did not differ from nontreated turf with respect to turf quality minima or average turf quality d⁻¹ based on AUPC (Table 2). The turf quality reductions caused by quinclorac and fenoxaprop (Table 2) are similar to that observed on creeping bentgrass in other studies (Carroll et al. 1992; Dernoeden et al. 2003; Henry and Hart 2004; Johnson 1994a).

All treatments, except those containing fenoxaprop or quinclorac, had 93% or greater creeping bentgrass cover at 9 WAIT (Table 2). The lower turf cover from fenoxaprop and quinclorac may be attributed to herbicide injury that reduced canopy density. Siduron applied at 8.9 to 18 kg ai ha⁻¹ did not cause creeping bentgrass canopy loss in two of three years in work done by Callahan (1972). In the third year of the Callahan (1972) study, siduron may have exacerbated creeping bentgrass loss by Pythium blight (*Pythium* spp.).

Across all three tolerance studies, metamifop, topramezone applied weekly, and both siduron programs were the safest treatments applied to creeping bentgrass putting greens. All fenoxaprop and quinclorac programs were deemed too injurious to creeping bentgrass greens. Although the exact treatment regimes used in these studies have not been evaluated elsewhere, our results generally align with other reports (Callahan 1972; Carroll et al. 1992; Johnson 1994a; Johnson and Carrow 1993; Parker et al. 2015) regarding creeping bentgrass response to the herbicides evaluated.

Weed Control from Low-Dose Herbicide Programs

Smooth crabgrass control. The treatment main effect was significant for smooth crabgrass and goosegrass control, cover, and shoot density at 9 WAIT ($P \le 0.0220$). The trial by treatment interaction was insignificant for all five response variables ($P \ge 0.0501$), and data for each variable were averaged over three site years (Table 3). All programs containing fenoxaprop, quinclorac, or siduron controlled smooth crabgrass 99 to 100% at 9 WAIT (Table 3). Topramezone controlled smooth crabgrass 69 to 77% with a slight improvement in control by weekly applications compared to monthly applications.

Researchers have observed that fenoxaprop programs applied at rates from 0.14 to 0.20 kg ha⁻¹ can control multi-tiller smooth crabgrass from 78 to 100%, but these rates are 8 to 12 times higher than our research and applied to smooth crabgrass mown at 2 cm or greater (Cox and Askew 2014; Derr 2002; Neal et al. 1990). Parker et al. (2015) conducted the only previous study that evaluated greens-height smooth crabgrass to fenoxaprop applied at 0.017 kg ha⁻¹. During this study, they observed that fenoxaprop applied three times at 3-wk intervals controlled smooth crabgrass 74%, which is significantly lower than our results from eight weekly treatments. Parker et al. (2015) also observed that metamifop applied twice at 0.20 kg ai ha⁻¹ controlled smooth crabgrass 99% which was similar to our results. Quinclorac applied three times at 0.37 kg ae ha⁻¹, nearly twice our highest rate, controlled 3- to 5-cm smooth crabgrass from 84 to 99% (Dernoeden et al. 2003). Quinclorac applied twice at 0.28 kg ha⁻¹ (similar to our 0.21 kg ha⁻¹ rate) controlled large crabgrass 79 to 99% (Johnson 1995).

Of 22 peer-reviewed papers reviewed that reported siduron use in turfgrass, only three assessed siduron use in mature turfgrass systems for smooth crabgrass control (Callahan and High 1990; Callahan et al. 1983; Murray et al. 1983) and none evaluated goosegrass or smooth crabgrass control in creeping bentgrass. Murray et al. (1983) found siduron at 11.2 kg ha⁻¹ applied to

Kentucky bluegrass lawn turf each April for eight years averaged 47 to 54% smooth crabgrass cover reduction 16 WAIT in the last three years. Callahan and High (1990) reported that siduron at 27 kg ha⁻¹ controlled smooth crabgrass 67 to 89% 12 WAIT in lawn-height bermudagrass. During spring establishment of tall fescue, siduron applied once or twice at a total rate load of 6.7 kg ha⁻¹ controlled smooth crabgrass from 4 to 60% late summer (Willis et al. 2006).

Topramezone at a total rate load of 0.012 kg ha⁻¹ divided into weekly and biweekly applications controlled smooth crabgrass 77 and 74, respectively 9 WAIT (Table 3). Elmore et al. (2012) reported that 50% control of greenhouse-grown smooth crabgrass requires 0.020 to 0.043 kg ha⁻¹ topramezone depending on nitrogen inputs. Brosnan et al. (2014) reported that 0.012 kg ha⁻¹ topramezone applied once to lawn-height tall fescue controlled smooth crabgrass 5 to 27% 9 WAT in Tennessee and Indiana. Although no published studies have evaluated topramezone applied to greens-height turf, comparing results of previous work to the current study suggest that either greens-height smooth crabgrass is easier to control or frequent, low-dose treatments are more effective than single, high-dose treatments. The later speculation is supported for mesotrione (Willis et al. 2007), a similar triketone herbicide to topramezone, where 0.028 kg ai ha⁻¹ applied twice controlled nimblewill as well as 280 g ha⁻¹ applied once. Smooth crabgrass cover and shoot density data mirror trends in smooth crabgrass control with the exception that all topramezone programs had equivalent cover and shoot densities, while the monthly program had slightly less control than the weekly program (Table 3).

Goosegrass control. All topramezone and fenoxaprop programs and the metamifop program controlled goosegrass 93 to 98% 9 WAIT and more than quinclorac or siduron programs (Table 3). Fenoxaprop applied at 0.20 kg ha⁻¹ failed to control any goosegrass in a Kentucky bluegrass golf fairway in Georgia (Johnson 1994b), while fenoxaprop applied at 0.10 kg ha⁻¹ controlled

goosegrass 40% on a creeping bentgrass green in Alabama (Parker et al. 2015). These previous reports show that fenoxaprop can harm goosegrass, especially at greens height, but fenoxaprop has not previously been evaluated in frequent, low-dose programs for goosegrass control as in the current study. Cox et al. (2017) observed that topramezone applied twice at 0.0061 kg ha⁻¹ controlled goosegrass 84 to 92%. This level of goosegrass control is similar to our results for all topramezone programs, while the weekly and biweekly topramezone rates are two to four times lower.

Quinclorac controlled goosegrass 0% at 9 WAIT (Table 3). Quinclorac can cause transient phytotoxicity but seldom controls goosegrass (Johnson 1994b). Siduron applied weekly at 5.6 kg ha⁻¹ or biweekly at 11 kg ha⁻¹ controlled goosegrass 36 to 39% (Table 3). Berry and Buchanan (1974) showed that siduron applied from 1.1 to 3.4 kg ha⁻¹ controlled goosegrass pre-emergently based on a 90% reduction in seedling counts 10 d after treatment to bare ground, while Fry et al. (1986) observed that siduron applied at 13.4 kg ha⁻¹ followed by a sequential application at 6.7 kg ha⁻¹ did not control any goosegrass during zoysiagrass establishment via plugs in Maryland. Kerr (1969) evaluated response of 118 grass species to rates of siduron and found that goosegrass and smooth crabgrass seedling growth was reduced 50% by siduron at 2.2 and 1.1 kg ha⁻¹, respectively, while >13 kg ha⁻¹ was required to reduce creeping bentgrass seedling growth equivalently. Kerr's work agrees with our research in that a large degree of selectivity exists between creeping bentgrass and the two weedy grasses, and that smooth crabgrass is more sensitive to siduron than goosegrass (Kerr 1969). As with smooth crabgrass, goosegrass cover and shoot density mirror the trends in goosegrass control (Table 3).

Creeping Bentgrass and Smooth Crabgrass Response to Siduron Rates

The treatment main effect was significant for creeping bentgrass cover, turf quality, smooth crabgrass control, smooth crabgrass cover, and smooth crabgrass density (P = 0.0172, P = 0.0034, P < 0.0001, P = 0.0152, P < 0.0001), and no response variable was dependent on trial ($P \ge 0.0523$) (Table 4). At 14 WAIT, siduron-treated plots averaged 93 to 97% creeping bentgrass cover compared to 59% cover in nontreated plots (Table 4). The poor cover evident in nontreated plots was caused by weed infestation. Increased creeping bentgrass cover in siduron treated plots led to a concomitant increase in average turf quality d^{-1} based on AUPC (Table 4).

Siduron applied five times biweekly at 3.4 kg ha⁻¹ controlled smooth crabgrass 96% and less than all other siduron rates, which controlled smooth crabgrass 99% (Table 4). Sidurontreated plots averaged 0.1 to 1.7% plot cover and between 0.75 and 18.5 smooth crabgrass plants m⁻², while the nontreated plots averaged 40% cover and 312 plants m⁻² (Table 4). This near equivalence in weed control across all siduron rates would have a substantial economic impact on weed management costs since current market value of siduron ranges from \$36 to \$49 kg⁻¹ active ingredient depending on product and supplier. Past research shows that siduron can be rather inconsistent for smooth or large crabgrass control, but none of these studies apply more than two applications of siduron per year (Hart et al. 2004; Willis et al. 2006), while we applied all four rates biweekly for a total of five applications. The general safety observed in these trials by creeping bentgrass to siduron is supported by the previous field studies as well as other published research (Callahan 1972; Johnson and Carrow 1993). Of the herbicides evaluated in this study, only siduron is currently registered for use on creeping bentgrass greens in the United States (Anonymous 2014). Siduron was shown to be safe to creeping bentgrass under a wide range of rates and effectively controls smooth crabgrass for the duration of a typical growing season in Virginia. Metamifop and topramezone also show promise for potential use on creeping bentgrass

greens for goosegrass control. These frequent, low-dose programs offer a novel solution for season-long smooth crabgrass and goosegrass control on creeping bentgrass greens.

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Table 1. Product name, manufacturer, common name, rates used, application intervals, adjuvant

Product name	Company	Common chemical name	Rate ^a	Application interval
			kg ha ⁻¹	wks
Acclaim Extra	Bayer Environmental Sciences, Cary, NC 27513	Fenoxaprop	0.018, 0.035	1, 2
Drive XLR8	BASF Corp., Research Triangle Park, NC 27709	Quinclorac	0.10, 0.21	1, 2
Dyne-Amic ^b	Helena Chemical Comp., Collierville, TN 38017	Modified vegetable oil	b	b
Induce ^b	Helena Chemical Comp., Collierville, TN 38017	Nonionic surfactant		
Pylex SC	BASF Corp., Research Triangle Park, NC 27709	Topramezone	0.0015,	1, 2, 4
			0.0031,	
			0.0061	
SAH-001	Summit Agro International, Tokyo, Japan	Metamifop	0.20	4
Tupersan 50WP	PBI Gordon Corp., Kansas City, MO 64101	Siduron	5.6, 13.5	1, 2
	1 -			

^aQuinclorac and topramezone expressed as kg ae ha⁻¹; fenoxaprop, metamifop, and siduron expressed as kg ai ha⁻¹

^bInduce was applied at 0.25% v/v with all applications that contained fenoxaprop or metamifop; Dyne-Amic was applied at 0.5% v/v with all applications that contained topramezone or quinclorac.

Table 2. Influence of herbicide treatment on maximum turf injury, average turf injury d⁻¹ based on area under the progress curve (AUPC), minimum turf quality, average turf quality AUPC d⁻¹ and final turf cover at 9 weeks after initial treatment (WAIT) averaged over three site years.

		No. applications	Turf injury	Turf	Turf quality	Turf	Turf cover
Treatment	Rate ^a	and frequency	maxima	injury	minima	quality	9 WAIT
	kg ha ¹	-	%	— AUPC d ⁻¹ —	— 1-9 —	—AUPC d ⁻¹ —	<u> </u>
Nontreated					6.3	6.7	93
Topramezone	0.0015	8 weekly	6.9	2.8	6.2	6.7	95
Topramezone	0.0031	4 biweekly	15	6.0	5.8	6.5	95
Topramezone	0.0061	2 monthly	38	16	5.2	6.5	95
Quinclorac	0.11	8 weekly	51	39	4.6	6.9	54
Quinclorac	0.21	4 biweekly	56	46	4.5	5.3	60
Fenoxaprop	0.018	8 weekly	54	43	4.2	5.1	46
Fenoxaprop	0.035	4 biweekly	74	63	3.7	4.6	63
Siduron	5.6	8 weekly	7.0	2.6	6.5	7.0	96
Siduron	11.2	4 biweekly	10	4.5	6.1	6.7	95
Metamifop	0.20	2 monthly	8	3.7	6.2	6.8	93
LSD (0.05)			3.6	2.6	0.3	1.2	5.2

^aTopramezone and quinclorac expressed as kg ae ha⁻¹; fenoxaprop and metamifop expressed as kg ai ha⁻¹

Table 3. Influence of herbicide treatment on smooth crabgrass and goosegrass control, cover, and shoot density at 9 weeks after initial treatment (WAIT) averaged over three site years.

		No. applications	Smooth crabgrass		Goosegrass			
Treatment	Rate ^a	and frequency	Control	Cover	Shoot density	Control	Cover	Shoot density
	kg ha ⁻¹	-	(%	—no. m ⁻² —	9	%	no. m ⁻²
Nontreated				29	1799		30	1049
Topramezone	0.0015	8 weekly	77	9.4	1020	98	0.7	66.40
Topramezone	0.0031	4 biweekly	74	11	1134	95	1.5	116.6
Topramezone	0.0061	2 monthly	69	14	1110	93	2.4	211.7
Quinclorac	0.11	8 weekly	100	0.0	0.000	0.0	43	1353
Quinclorac	0.21	4 biweekly	100	0.0	0.000	0.0	42	1478
Fenoxaprop	0.018	8 weekly	100	0.0	5.600	99	0.2	48.40
Fenoxaprop	0.035	4 biweekly	99	0.1	11.20	97	0.8	37.70
Siduron	5.6	8 weekly	100	0.0	0.000	39	23	986.5
Siduron	11.2	4 biweekly	99	0.1	7.500	36	31	1096
Metamifop	0.20	2 monthly	99	0.3	82.20	98	0.6	116.6
LSD (0.05)				8.2	605.9	15	11	246.7

^aTopramezone and quinclorac expressed as kg ae ha⁻¹; fenoxaprop and metamifop expressed as kg ai ha⁻¹

Table 4. Influence of siduron rate on creeping bentgrass cover 14 wk after initial treatment (WAIT), average turf quality d⁻¹ based on area under the progress curve (AUPC), and smooth crabgrass control, cover, and density at 14 WAIT averaged over two years.

		Creeping bentgrass	Turf	Smooth crabgrass		
Treatment	Rate	cover	quality	Control	Cover	Plant density
	kg ai ha ⁻¹	%	—AUPC d ⁻¹ —	%	%	— no. m ⁻² —
Nontreated		59	5.2		40	312
Siduron	3.4	93	5.9	96	1.7	18.5
Siduron	6.7	94	6.3	99	0.3	3.00
Siduron	10	96	6.0	99	0.1	0.88
Siduron	13	97	6.3	99	0.1	0.75
LSD (0.05)		8.1	0.5	1.6	7.3	47.9