POA TRIVIALIS: PHYSIOLOGICAL AND PATHOLOGICAL COMPONENTS OF SUMMER DECLINE, AND CULTURAL, SELECTIVE, AND NON-SELECTIVE CONTROL METHODS

by

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B.S., Kansas State University, 2008
M.S., Kansas State University, 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture, Forestry, and Recreation Resources
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Abstract

Rough bluegrass (RBG, *Poa trivialis* L.) is a difficult-to-control weed that commonly infests cool-season turfgrass swards after movement of vegetative propagules or contamination from seed lots. Rough bluegrass is less tolerant of heat stress than desirable cool-season species such as tall fescue (TF, *Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.), and often declines during mid-summer due to biotic or abiotic stresses. The objectives of these 2011-2013 controlled environment and field experiments were to: 1) observe growth and physiological differences between 'Laser' and 'Pulsar' RBG and TF; 2) differentiate between physiological and pathological contributors to RBG decline; 3) determine the effects of TF seeding rate and mowing height on TF/RBG establishment when RBG is a seed contaminant; 4) evaluate herbicide combinations for selective RBG control; and 5) evaluate seasonal timing of glyphosate for nonselective RBG control. Tall fescue was less affected by elevated temperature than RBG. At 35°C, Laser and Pulsar experienced similar reductions in quality, gross photosynthesis (Pg), shoot and root biomass, and root length density compared to when grown at 23°C, but maximum electrolyte leakage was greater for Pulsar (63%) than for Laser (49%). Cell membrane thermostability could contribute to the better heat tolerance of Laser RBG. Evaluation of RBG foliage and roots did not reveal a fungal pathogen associated with RBG decline. Still, repeated applications of azoxystrobin (610 g a.i. ha^{-1}) or pyraclostrobin (556 g a.i. ha^{-1}) increased RBG quality, cover, and Pg during summer compared to untreated RBG, possibly due to poorly understood non-target physiological effects of the fungicides. Mowing TF at 7.6 or 11.4 cm reduced RBG incidence up to 57% compared to mowing at 3.8 cm. Tall fescue seeding rate had no effect on RBG incidence. Several herbicides and herbicide combinations resulted in some RBG injury in the field, but bispyribac-sodium was the only treatment that provided RBG control (16 to 92%) in Manhattan, KS; Hutchinson, KS; and Mead, NE. Spring-applied glyphosate resulted in the lowest RBG coverage (1 to 31%) among field studies in Manhattan and Mead, followed by late-summer applications (6 to 58%), and mid-summer applications (9 to 86%).

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Dedication

I dedicate this work to my loving wife, Sally. Thank you for your unconditional love and support. This journey would have been far more difficult without you in my life.

Chapter 1 - Review of Literature

Rough Bluegrass Origin and Use

Rough bluegrass (*Poa trivialis* L.), also commonly known as roughstalk bluegrass, rough meadowgrass, and roughstalk meadowgrass (Beard, 1973), is a C_3 cool-season perennial turfgrass species that is native to northern Europe, temperate Asia, and northern Africa (Hubbard, 1954). Rough bluegrass was likely originally introduced to North America as a contaminant in Kentucky bluegrass (*Poa pratensis* L.) seed (Hurley, 2003). Rough bluegrass forms a yellow-green, moderately fine-textured, medium dense turf that produces leafy stolons (Beard, 1973; Hurley, 2003). The species grows aggressively under suitable conditions, often segregating into distinct patches that do not blend well in mixed stands of other cool-season turfgrass species (Beard, 1973; Fry and Huang, 2004; Hurley, 2003). Still, rough bluegrass may go unnoticed in mixed stands with other cool-season turfgrasses until decline during summer months (Fry and Huang, 2004) due to its sensitivity to heat and drought stresses (Beard, 1973), and potential susceptibility to turf diseases including brown patch (*Rhizoctonia solani* Kuhn), dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett), and pythium blight (*Pythium spp.*) (Hurley, 2003). Rough bluegrass is cross-pollinated and has a significant amount of genetic variability among biotypes (Beard, 1973; Hurley, 2003). Older, common-type cultivars (e.g. 'Danish common,' 'Dasas,' 'Polis,' and 'Indo') were produced in Europe and are light in color, and form a loose-growing sod with a high vertical growth rate. There are now several improved cultivars (e.g. 'Cypress,' 'Sabre,' 'Laser,' 'Winterplay,' 'Darkhorse') that were bred to be lower growing, darker green turfs that produce a denser sod with improved disease resistance (Hurley, 2003).

Commercially, rough bluegrass is recommended for use in shaded, moist lawns and is often used for winter overseeding programs on bermudagrass (*Cynodon spp.*) turfs in southern climates (Fry and Huang, 2004; Hurley, 2003). Rough bluegrass responds more to irrigation than any other cultural practice (Beard, 1973), and, despite its sensitivity to heat, has survived summer months in overseeded bermudagrass putting greens in Florida if water is not limiting (Hurley, 2003).

Rough Bluegrass Physiology and Growth

Rough bluegrass has excellent low temperature hardiness and its seed can germinate and grow at low temperatures (Hurley, 2003). However, rough bluegrass is quite susceptible to heat and drought stresses due to its fibrous, shallow root system. It is well adapted to cool, damp, shaded locations not subjected to concentrated traffic (Beard, 1973; Hurley, 2003). Many commonly used cool-season turfgrass species are less sensitive to heat stress than rough bluegrass (Sifers and Beard, 1993). Still, high temperature stress is the main factor causing leaf senescence and physiological damage of cool-season turfgrasses (Cross et al., 2013; Xu and Huang, 2009) and occurs at temperatures greater than 30°C (Fry and Huang, 2004). Rates of photosynthesis are more inhibited by high temperature stress than respiratory rates. This leads to an imbalance whereby carbon used in respiration exceeds that provided by photosynthesis, ultimately depleting carbohydrate reserves (Taiz and Zeiger, 2010). The reduced photosynthetic capacity of plants exposed to heat stress has been associated with reductions in photochemical (e.g. carotenoids, chlorophyll a and b) efficiency of photosystem II, the interruption of electron transport, and reduced CO_2 fixation and assimilation resulting from reduced ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) activity (Berry and Björkman, 1980; Liu and Huang, 2008, Xu and Huang, 2000). Additionally, the induction of free radicals such as hydrogen peroxide (H_2O_2) during heat stress results in lipid peroxidation, ultimately degrading cell membranes and possibly inhibiting photosynthesis and respiration (Fry and Huang, 2004).

High temperature stress can also greatly affect turfgrass rooting. The fine root system is the primary pathway for water and nutrient absorption in vascular plants and root system maintenance requires a large allocation of carbon, often at the expense of new shoot growth (Eissenstat, 1992). Increased soil temperatures also adversely affect shoots maintained at optimal temperatures. Air temperature maintained at 20°C and root zone temperatures in excess of 23°C have been shown to be detrimental to root activities and net photosynthesis (Pote et al., 2006). Likewise, root zone temperatures of 25°C or greater may decrease cytokinin content, root number, and root biomass; and soil temperatures of 35°C can result in decreased rooting depth and overall turf quality (Pote et al., 2006). Similarly, Wang et al. (2003) observed decreased antioxidant and cytokinin content in shoots of creeping bentgrass (*Agrostis stolonifera* L.) maintained at 20°C when soil temperatures were 25°C or greater.

Several researchers have demonstrated the sensitivity of rough bluegrass to high temperature stress (Carroll and Welton, 1937; Loveys et al., 2002; Rutledge et al., 2012a; Rutledge et al., 2012b; Sifers and Beard, 1993; Watschke et al., 1973). For example, rough bluegrass maintains more fully open stomata during daylight hours compared to other cool-season species, suggesting increased transpiration of rough bluegrass (Carroll and Welton, 1937). Similarly, Watschke et al. (1973) and Loveys et al. (2002) observed increases in rough bluegrass respiration with increasing temperature, and Watschke et al. (1973) observed a 50% decrease in rough bluegrass photosynthesis at 35°C compared to 23°C.

More recently, researchers at Purdue observed stark physiological differences between creeping bentgrass and rough bluegrass exposed to supraoptimal temperatures (Rutledge et al., 2012b). In a controlled environment study, creeping bentgrass exhibited better turf quality at 28 and 35 days after heat treatments began. Rough bluegrass shoot growth declined after only 11 days at 33°C. After 35 days at 33°C, amino acid concentrations in rough bluegrass and creeping bentgrass shoots increased 223 and 64%, respectively, compared to plants at 23°C. Perhaps most interestingly, rough bluegrass roots maintained higher total nonstructural carbohydrate and fructan concentrations than creeping bentgrass roots, indicating that rough bluegrass may not have been able to hydrolyze fructan to simple sugars for metabolic activity (Rutledge et al., 2012b). In a separate study, Rutledge et al. (2012a) observed total nonstructural carbohydrate concentrations in 'L-93' creeping bentgrass shoots remained unchanged in mid- to late-summer, while concentrations in Laser and 'Pulsar' rough bluegrass shoots decreased 18 to 26%, possibly due to degradation, metabolism, or translocation to stolons. Protein and amino acid concentrations followed a similar trend. Furthermore, total nonstructural carbohydrate concentrations in shoots of Laser and Pulsar increased 19 and 29%, respectively, during fall along with a respective 23 and 31% decrease in stolons, suggesting that the rapid decline of rough bluegrass during high temperature exposure is a survival mechanism whereby stolons are used as survival structures until favorable growing conditions return (Rutledge et al., 2012a).

Rutledge et al. (2012b) selected Laser and Pulsar rough bluegrass for their experiment as relatively heat-tolerant and heat-sensitive cultivars, respectively, but they did not observe differences in turf quality, clipping yields, chlorophyll content, electrolyte leakage, root dry weight, root viability, or amino acid, total nonstructural carbohydrate, fructan, glucose, or protein concentrations in shoots/roots between the cultivars when exposed to elevated temperatures.

Electrolyte leakage is a measure of cell membrane thermostability that is commonly used to evaluate relative heat tolerance of plants (Jiang and Huang, 2001; Marcum, 1998; Su et al., 2009; Wallner et al., 1982). While tall fescue (*Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.) is commonly accepted to be more heat tolerant than other cool-season species, in part due to its deep root system that maintains an adequate water supply for transpirational cooling (Fry and Huang, 2004), Wallner et al. (1982) reported that tall fescue is not more heat tolerant than perennial ryegrass (*Lolium perenne* L.) *in vitro* according to electrolyte leakage estimates. Conversely, Jiang and Huang (2001) reported that electrolyte leakage increased more rapidly for perennial ryegrass than for tall fescue when exposed to heat stress alone, or in combination with drought stress. More recently, Cross et al. (2013) tested 24 tall fescue genotypes in a growth chamber study to compare “summer stress-tolerant” and “summer stress-sensitive” selections. There were few differences among selections under drought stress or a combination of heat and drought stresses. However, when selections were exposed only to heat stress, summer stress-tolerant selections had better turf quality, higher photochemical efficiency, and less electrolyte leakage compared to summer stress-sensitive selections indicating better heat tolerance. Interestingly, one TF clone (designated TF-3) was a top-performing selection under heat stress, but was among the worst selections under drought stress or a combination of heat and drought stress. Because TF-3 had very low soil volumetric water content under optimal conditions and under all combinations of heat and drought stresses, the researchers concluded that its heat tolerance resulted from a high level of transpirational cooling due to high water use (Cross et al., 2013).

Since there is still no empirical explanation for the differences in relative heat tolerance between Laser and Pulsar, further examination of differences in EL, as well as other stress tolerance parameters, between the two cultivars and a heat tolerant turf species is warranted.

Pathogenic Contribution to Summer Decline

While heat and drought stresses are likely the main instigators in rough bluegrass summer decline, there is some indication that disease susceptibility may play a larger role than previously thought. Recently, rough bluegrass subjected to applications of azoxystrobin or fungicide mixtures containing azoxystrobin at two-week intervals beginning in May maintained quality and cover during summer months in Indiana compared to rough bluegrass not treated with azoxystrobin, or treated with azoxystrobin beginning after decline had ensued in July (Weisenberger and Reicher, 2006 and 2007). These results indicate that summer rough bluegrass decline may be due, in part, to one or more pathogens. Azoxystrobin is a strobilurin (Q_oI) fungicide (Fungicide Resistance Action Committee [FRAC] Code 11). In fungi, strobilurin fungicides inhibit mitochondrial respiration at the Q_o site of cytochrome *b*, blocking electron transfer between cytochrome *b* and c_1, thus preventing ATP synthesis (Bartlett et al., 2002). While strobilurin fungicides provide control of diseases caused by ascomycetous, basidiomycetous, and oomycetous fungi, many have also been associated with increased yield and quality in various plants even with trivial differences in disease control (Bartlett et al., 2002). For example, studies have shown treatment with azoxystrobin, kresoxim-methyl, trifloxystrobin, picoxystrobin, or pyraclostrobin have positively affected yield and grain size in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), by influencing the maintenance of green leaf area of crops until late in the season, thereby maximizing the grain-filling period (Bartlett et al., 2002). On the other hand, Wrather et al. (2004) observed that treatment with azoxystrobin resulted in lower quality soybean [*Glycine max* (L.) Merr.] seed. Applications of kresoxim-methyl or pyraclostrobin to wheat under drought stress evoked similar plant responses to low quantities of auxins reducing ethylene activity and increasing cytokinin content and antioxidant activity (Grossmann and Retzlaff, 1997; Köhle el al., 2002). More recently, researchers in Tennessee examined the effects of strobilurin fungicides on creeping bentgrass under heat and/or drought stress (Brosnan et al., 2010). No changes in turfgrass quality were observed after fungicide applications, but azoxystrobin reduced visual root length and total root biomass of ‘Penncross’ creeping bentgrass, and total root length, root length density, and total root biomass of ‘Penn A-1’ creeping bentgrass compared to untreated turf at 27°C under well-watered conditions. Conversely, treatment with pyraclostrobin increased visual root length for both cultivars and also increased total root length, root surface area, root length density, root volume, and root biomass for Penn A-1 compared to untreated turf at 27°C and irrigated to prevent leaf wilt.

Considering recent research, it is unclear if the improved rough bluegrass quality during summer observed by Weisenberger and Reicher (2006 and 2007) resulted from disease control from preventative fungicide applications of azoxystrobin, combinations including azoxystrobin, or from potential non-target effects of strobilurin fungicides. More investigation is needed.

Rough Bluegrass Control During Establishment

After its introduction from Europe, rough bluegrass was both inadvertently and intentionally seeded throughout much of North America, leading to its naturalization (Hurley, 2003). Currently, naturalized populations are thought to spread vegetatively during routine cultural practices (e.g. aeration), while improved varieties with fine texture and relatively dark green color are likely introduced directly from seed lots (Levy, 1998; Reicher et al., 2011). Weed control in seed production has become more difficult since 1990, when a mandatory change from burning to mechanical removal of post harvest residue was initiated (Mueller-Warrant, 1990; Mueller-Warrant and Rosato, 2005). In 1996, Levy (1998) tested 90 creeping bentgrass seed samples from 10 seed companies and found that 30% of seed lots contained rough bluegrass seed. Following this study, seed producers moved creeping bentgrass seed production areas away from rough bluegrass production areas and improved sanitation procedures (Reicher et al., 2011). Nonetheless, rough bluegrass contamination remains a major concern. In 2008, Reicher et al. (2011) sampled 37 cultivars/blends of creeping bentgrass from 10 distributors from five Midwestern states, the majority of which were certified. Rough bluegrass was detected in 8 of 72 seed lots.

Herbicidal Control Before and After Seeding

On golf courses, bispyribac-sodium (Velocity, Valent U.S.A. Corporation, Walnut Creek, CA) can be a useful rough bluegrass control tool during establishment, especially with the likelihood of seed contamination. Bispyribac-sodium is a pyrimidinylthiobenzoic acid herbicide that inhibits acetolactate synthase (ALS), blocking amino acid synthesis in sensitive plants (Lycan and Hart, 2006; Senseman, 2007). Rutledge et al. (2010b) made a single application of bispyribac-sodium at 18, 37, 55, or 74 g a.i. ha^{-1} at 7, 14, 21, or 28 days after creeping bentgrass emergence in spring and fall. Rutledge et al. concluded that bispyribac-sodium may be applied to spring- and fall-seeded creeping bentgrass as early as seven days after emergence at 55 and 74 g a.i. ha^{-1} or less, respectively. In spring, applications were most effective at or exceeding 37 g a.i. ha^{-1} at 7 or 14 days after creeping bentgrass emergence.

Bispyribac-sodium can also be applied for rough bluegrass control before seeding. In a field study in New Jersey, bispyribac-sodium applied at 148 or 296 g a.i. ha^{-1} did not reduce ground cover of desirable species (creeping bentgrass, Kentucky bluegrass, or perennial ryegrass) when applied two or more weeks before seeding (Lycan and Hart, 2006). Similarly in a three-year study in Indiana from 2006 to 2008, Rutledge et al. (2010a) applied bispyribac-sodium four times at a two-week interval at 37, 56, or 74 g a.i. ha^{-1} and then seeded half of each plot with creeping bentgrass at 49 kg ha^{-1} two weeks following the final herbicide treatment. In 2006 and 2007, all bispyribac-sodium treatments reduced rough bluegrass cover to less than 27% compared to a minimum of 66% rough bluegrass cover in untreated plots by 46 weeks after seeding. However, due to cooler summer temperatures in 2008, herbicide treatments were not as effective as in 2006 and 2007 and rough bluegrass cover in herbicide-treated plots was not different from untreated plots by 46 weeks after seeding. By 46 weeks after seeding in 2006 and 2007, interseeding with creeping bentgrass resulted in 69 and 85% creeping bentgrass cover, respectively, compared to 39 and 15%, respectively, in unseeded plots. Similar to herbicide treatments, interseeding had no effect on creeping bentgrass cover in 2008 (Rutledge et al., 2010a). Interseeding with desired species following bispyribac-sodium application is an important strategy for long-term rough bluegrass control.

Cultural Control in Tall Fescue

Rough bluegrass seed contamination is also a concern in tall fescue sports fields and residential lawns in the transition zone. Even though rough bluegrass seed contamination has not been empirically confirmed in tall fescue seed lots as it has been in creeping bentgrass seed lots, it is generally accepted that rough bluegrass is introduced into tall fescue lawns and sports fields as a seed contaminant. In fact, tall fescue seed yields decrease with increasing rough bluegrass ground cover in production fields (Mueller-Warrant and Rosato, 2005), potentially validating contamination concerns. Once established, glyphosate is currently the only herbicidal control option in residential lawns and sports fields. During establishment, rough bluegrass is very competitive and has been shown to out compete perennial ryegrass. When intentionally seeded in mixtures, rough bluegrass decreased the tillering of perennial ryegrass by up to 30% (Haggar, 1979). In a separate study, rough bluegrass had a higher relative growth rate than perennial ryegrass from 25 to 81 days after emergence (Vartha, 1973). Despite rough bluegrass's aggressive growth habit, it may be possible to favor tall fescue over rough bluegrass during establishment by altering seeding rates and/or mowing heights. For example, higher mowing heights favor tall fescue over bermudagrass [*Cynodon dactylon* L. (Pers.)] and smooth crabgrass

[*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.]. Mowing tall fescue at 6 cm reduced bermudagrass encroachment over a season compared to mowing at 2 cm (Brede, 1992), while mowing tall fescue at 9 cm significantly reduced the amount of smooth crabgrass establishment in a season compared to mowing at 3 cm (Dernoeden et al., 1993). Similarly, Voigt et al. (2001) observed more crabgrass (*D. spp.*) in tall fescue mowed at 2.5 cm compared to that mowed at 5.1 or 7.6 cm. Higher initial mowing heights also favor perennial ryegrass over Kentucky bluegrass when seeded in mixtures. Brede and Duich (1984) observed that perennial ryegrass/Kentucky bluegrass seed mixtures mowed at 3.8 cm required no less than 95% Kentucky bluegrass seed to produce a 50:50 stand two months after seeding, wheras mixtures mowed at 1.3 cm required only 50 to 75% Kentucky bluegrass for a 50:50 stand. Further information is needed to optimize seeding rates and mowing height in tall fescue stands to minimize rough bluegrass colonization.

Postemergence Rough Bluegrass Control with Herbicides

Few herbicides are effective for the selective control of rough bluegrass in cool-season grasses. Fenoxaprop applied at 0.28 kg a.i. ha^{-1} reduced rough bluegrass in perennial ryegrass fields in Oregon (Mueller-Warrant, 1990), but there are no other reports of rough bluegrass control with fenoxaprop. Neal and Senesac (1993) observed up to 20% injury of rough bluegrass with application of quinclorac (0.8 kg a.i. ha^{-1}) + 2,4-dichlorophenoxy acetic acid (1.1 kg a.i. ha^{-1}) by six weeks after treatment, but rough bluegrass recovered by 12 weeks after treatment. In a field study in Georgia, applications of oryzalin, oryzalin + benefin, and oryzalin + oxyfluorfen were severely injurious to overseeded Laser rough bluegrass in a 'Tifway' bermudagrass [*Cynodon transvaalensis* Burtt-Davy × *C. dactylon* (L.) Pers] putting green and influenced the rate of summer transition back to bermudagrass (Johnson, 1998). However, preemergence herbicides will not control perennial weeds like rough bluegrass once established, and oryzalin is injurious to desirable cool-season turf species. Rough bluegrass also tolerates fenarimol, which is a demethylation inhibiting (DMI) fungicide that inhibits ergosterol synthesis in fungal cell membranes, and has preemergence herbicidal activity on annual bluegrass (Johnson, 1994).

There may be potential for rough bluegrass suppression with plant growth regulators. Monthly applications of trinexapac-ethyl at 0.19 kg a.i. ha^{-1} resulted in a 75% rough bluegrass reduction after seeding a mixture of creeping bentgrass, annual bluegrass, and rough bluegrass in a field study in Ohio (Bell et al., 1999). Paclobutrazol is more commonly used for annual bluegrass suppression on golf course fairways and putting greens (Baldwin and Brede, 2011; McCullough et al., 2005), but it is unclear if paclobutrazol suppresses rough bluegrass.

Bispyribac-sodium

The most successful selective rough bluegrass control has come from applications of bispyribac-sodium or sulfosulfuron (Certainty, Monsanto Co., St. Louis, MO). Sulfosulfuron is no longer labeled for use in cool season turf (Anonymous, 2012a). Morton et al. (2007) tested several rates and timings of bispyribac-sodium, and found that four applications at 56 or 74 g a.i. ha^{-1} at two-week intervals reduced rough bluegrass by 85% or more compared to untreated plots 12 weeks after initial treatment. All rates (37, 56, 74, and 114 g a.i. ha^{-1}) caused phytotoxicity in desired species, but turf recovered by two weeks after treatment. The researchers also noted that efficacy of bispyribac-sodium increased at warmer temperatures (~24 to 30°C). Askew et al. (2004) also observed increased efficacy of bispyribac-sodium when applications began at warmer times of the year in Virginia. Three applications of bispyribac-sodium at 74 g a.i. ha^{-1} beginning in June, August, and September reduced rough bluegrass cover 93, 95, and 31%, respectively, by 10 weeks after initial treatment, while applications at 37 g a.i. per ha^{-1} only reduced rough bluegrass 88, 48, and 11%, respectively. In two separate field experiments in New Jersey, bispyribac-sodium applied two (37, 74, or 111 g a.i. ha^{-1}) or three (37 or 74 g a.i. ha^{-1}) times at approximately three week intervals in two consecutive seasons beginning in June reduced rough bluegrass cover to 2% or less by August of the second season in both experiments with minimal phytotoxicity to creeping bentgrass (McCullough and Hart, 2011). Untreated plots averaged 9 and 4% cover at this time, respectively, and all bispyribac-sodium treatments had less rough bluegrass cover than untreated plots. There were no differences among rates or timings. However, by October in both experiments rough bluegrass had recovered in all bispyribac-sodium-treated plots and was not different from untreated. McCullough and Hart (2011) suggest that dormancy responses of rough bluegrass following applications likely limited translocation of herbicide to stems and roots, ultimately limiting control.

Observed differences in rough bluegrass control with bispyribac-sodium may be due, in part, to genetic variation within the species. The efficacy of bispyribac-sodium was not different among eight cultivars of rough bluegrass (‘Bariviera’, Laser, ‘Proam’, Pulsar, ‘Racehorse’, ‘Sabre II’, ‘Sun-Up’, and ‘Winterlinks’) when mowed at 1.25 cm in Indiana (Morton et al., 2009). However, Laser and Bariviera seemed to be more susceptible, and Pulsar less susceptible, to bispyribac-sodium than other cultivars when mowed at 5.0 cm.

Amicarbazone and Mesotrione

Even though bispyribac-sodium can be effective for rough bluegrass control, its use is limited to golf course and sod farm turf (Anonymous, 2010). More control options are needed. Amicarbazone (Xonerate, Arysta LifeScience, Cary, NC) and mesotrione (Tenacity, Syngenta Crop Protection, Greensboro, NC) are selective postemergence herbicides that are labeled for use in many turfgrass sites. Amicarbazone is labeled for use on golf courses, sod farms, residential and commercial turf sites, park and recreation areas, school grounds and other turf areas (Anonymous, 2012b). Mesotrione is labeled for use on golf courses, sod farms, athletic fields, parks, residential and commercial properties, cemeteries, airports, and lawns (Anonymous, 2011). In sensitive plants, amicarbazone inhibits photosystem II by blocking electron transport, whereas mesotrione is a *p*-hydroxyphenylpyruvate (HPPD)-inhibiting herbicide (Elmore et al., 2013). Recently, researchers have shown that amicarbazone and mesotrione more effectively control annual bluegrass (*Poa annua* L.) when tank-mixed than when either product is applied alone (Elmore et al., 2013). Elmore et al. (2013) explain that the synergistic effects of combinations of amicarbazone and mesotrione result from coinciding increased production of toxic singlet oxygen (amicarbazone) and decreased singlet oxygen quenching due to inhibited carotenoid production (mesotrione). Amicarbazone and mesotrione are not currently labeled for rough bluegrass control, but there is interest in evaluating the efficacy of the products, especially when applied in combination.

Nonselective Control

Currently, nonselective herbicides are often the only option for rough bluegrass control in sports fields and residential lawns. However, it is unclear if control varies with the seasonal timing of application as it often does with bispyribac-sodium (Askew et al., 2004; Morton et al., 2007; Rutledge et al., 2010a). Rough bluegrass has been anecdotally reported to persist in sites which have been renovated with use of glyphosate before fall seeding. Spring applications of imazapic plus glyphosate are more effective controlling Kentucky bluegrass compared to summer or fall applications (Adkins and Barnes, 2013), but the effects of seasonal timing of glyphosate application on rough bluegrass control are not known.

Objectives

In summary, relative differences in phenotypic heat tolerance among rough bluegrass cultivars are poorly understood. It is also unclear if turfgrass diseases contribute significantly to summer rough bluegrass decline, or if abiotic stresses are solely responsible. Rough bluegrass is difficult to control in cool-season turf. Seed contamination may result in the unintended presence of rough bluegrass in tall fescue swards, and further information is needed to culturally minimize rough bluegrass colonization during establishment. Selective postemergence herbicides are limited for rough bluegrass control in cool-season turf, especially in sites other than golf courses and sod farms. Synergistic herbicide combinations that selectively control annual bluegrass in cool-season turfgrasses may have efficacy against rough bluegrass. Lastly, nonselective herbicides are currently the most reliable rough bluegrass control option in cool-season turfgrasses, but the effect of seasonal timing of nonselective herbicide applications on rough bluegrass control has not been confirmed empirically. Therefore, my objectives were to: 1) observe growth and physiological differences between 'Laser' and 'Pulsar' rough bluegrass and tall fescue at elevated temperatures; 2) differentiate between physiological and pathological contributors to rough bluegrass decline; 3) determine the effects of tall fescue seeding rate and mowing height on tall fescue establishment and rough bluegrass encroachment when rough bluegrass is a seed contaminant; 4) evaluate herbicide combinations for selective rough bluegrass control; and 5) evaluate seasonal timing of glyphosate for nonselective rough bluegrass control.

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Chapter 2 - Growth and Metabolic Responses of Rough Bluegrass Cultivars and Tall Fescue to Elevated Temperatures

Abstract

Rough bluegrass (RBG, *Poa trivialis* L.) is a weed in other cool-season turfs because it declines in quality during prolonged high temperatures, often leaving unsightly patches. Rough bluegrass cultivars differ in heat tolerance, but growth and physiological factors in response to heat are poorly understood. The goal was to observe growth and physiological differences between two RBG cultivars and tall fescue (TF, *Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.), which has superior heat tolerance among cool-season grasses. Four months after seeding, pots of ‘Laser’ and ‘Pulsar’ RBG and ‘Second Millennium’ TF were subjected to optimal (23°C day/19°C night) or supraoptimal (35/29°C) temperatures for 35 days. At 35°C, Laser had unacceptable quality at 7 days after induction (DAI) of heat stress and Pulsar had unacceptable quality at 14 DAI, with no differences between the two thereafter. Gross photosynthesis (Pg) of Laser, Pulsar, and TF at 35°C was significantly lower than that of turfgrasses at 23°C by 0, 7, and 14 DAI, respectively, with a corresponding 36, 36, and 26 % reduction in Pg at 35°C by 35 DAI. Maximum electrolyte leakage (EL) values at 35°C were: Laser (49%), Pulsar (63%), and TF (24%). Laser and Pulsar both accumulated less shoot biomass at 35°C than TF. Root biomass and root length density (RLD) of Laser and Pulsar at 35°C were reduced compared to 23°C, whereas root biomass and RLD of TF was similar at 35 and 23°C. The root decline of Laser and Pulsar from 23 to 35°C likely contributed to reduced turf quality and Pg of RBG at elevated temperatures, but the lack of rooting differences between Laser and Pulsar with significant differences in EL between the two indicate that differences in cell membrane thermostability could play a significant role in heat tolerance among RBG cultivars.

Introduction

Rough bluegrass (RBG, *Poa trivialis* L.) is a perennial cool-season species that often declines during summer due to sensitivity to heat and drought stresses (Beard, 1973). Many commonly used cool-season turfgrass species are less sensitive to heat stress than RBG (Sifers and Beard, 1993), making RBG a weed in cultivated turfgrass systems. High temperature stress is the main factor causing leaf senescence and physiological damage of cool-season turfgrasses (Cross et al., 2013; Xu and Huang, 2009) and occurs at temperatures greater than 30°C (Fry and Huang, 2004). Rates of photosynthesis are more inhibited by high temperature stress than respiratory rates, resulting in an imbalance whereby carbon used by respiration exceeds that provided by photosynthesis, ultimately depleting carbohydrate reserves (Taiz and Zeiger, 2010). The reduced photosynthetic capacity of plants exposed to heat stress has been associated with reductions in photochemical (e.g. carotenoids, chlorophyll a and b) efficiency of photosystem II, the interruption of electron transport, and reduced CO_2 fixation and assimilation resulting from reduced ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) activity (Berry and Björkman, 1980; Liu and Huang, 2008, Xu and Huang, 2000a). Additionally, the induction of free radicals such as hydrogen peroxide (H_2O_2) during heat stress results in lipid peroxidation, ultimately degrading cell membranes and possibly inhibiting photosynthesis and respiration (Fry and Huang, 2004).

While the increased sensitivity of RBG to high temperature stress compared to other turfgrass species has been associated with increased respiration and/or decreased photosynthesis (Carroll and Welton, 1937; Loveys et al., 2002; Watschke et al., 1973), Rutledge et al. (2012b) recently observed differences in creeping bentgrass (*Agrostis stolonifera* L.) and RBG shoot amino acid concentrations and root nonstructural carbohydrate concentrations during heat stress. After 35 days at 33°C, RBG shoot amino acid concentrations increased 223% compared to plants at 23°C. Rough bluegrass roots also maintained higher total nonstructural carbohydrate and fructan concentrations than creeping bentgrass roots, indicating that RBG may not have been able to hydrolyze fructan to simple sugars for metabolic activity (Rutledge et al., 2012b). In a separate study, Rutledge et al. (2012a) observed that total nonstructural carbohydrate concentrations in 'Laser' and 'Pulsar' RBG shoots in midsummer decreased 18 to 26%, possibly due to degradation, metabolism, or translocation to stolons. In fall, the researchers observed an influx of nonstructural carbohydrates to shoots, presumably from stolons, suggesting that stolons function as survival structures during high temperature stress.

Rough bluegrass cultivars/biotypes differ with regard to heat tolerance, but physiological/morphological differences among cultivars have not been identified. Rutledge et al. (2012b) selected Laser and Pulsar RBG for their experiment as relatively heat-tolerant and heat-sensitive cultivars, respectively, but they did not observe differences in turf quality, clipping yields, chlorophyll content, electrolyte leakage (EL), root dry weight, root viability, or amino acid, total nonstructural carbohydrate, fructan, glucose, or protein concentrations in shoots/roots between the cultivars when exposed to elevated temperatures. Electrolyte leakage is a measure of cell membrane thermostability that is commonly used to evaluate relative heat tolerance of plants (Jiang and Huang, 2001; Marcum, 1998; Su et al., 2009; Wallner et al., 1982). While tall fescue (TF, *Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.) is commonly accepted to be more heat tolerant than other cool-season species, in part due to its deep root system that maintains an adequate water supply for transpirational cooling (Fry and Huang, 2004), Wallner et al. (1982) reported that TF is not more heat tolerant than perennial ryegrass (*Lolium perenne* L.) *in vitro* according to EL estimates. Conversely, Jiang and Huang (2001) reported that EL increased more rapidly for perennial ryegrass than for TF when exposed to heat stress alone, or in combination with drought stress. More recently, Cross et al. (2013) tested 24 TF genotypes in a growth chamber study to compare summer stress-tolerant and summer stress-sensitive selections. There were few differences among selections under drought stress or a combination of heat and drought stresses. However, when selections were exposed only to heat stress, summer stress-tolerant selections had better turf quality, higher photochemical efficiency, and less EL compared to summer stress-sensitive selections indicating better heat tolerance. Interestingly, one TF clone (designated TF-3) was a top-performing selection under heat stress, but was among the worst selections under drought stress or a combination of heat and drought stress. Because TF-3 had very low soil volumetric water content under control conditions and under all combinations of heat and drought stresses, the researchers concluded that its heat tolerance resulted from a high level of transpirational cooling due to high water use (Cross et al., 2013).

Since there is still no empirical explanation for the differences in relative heat tolerance between Laser and Pulsar, further examination of differences in EL, as well as other stress tolerance parameters, between the two cultivars and a heat tolerant turf species is warranted. Therefore, the objective was to evaluate the heat tolerance of Laser, Pulsar, and TF by comparing differences in gross photosynthesis (Pg), clipping production, EL, aboveground biomass production, root length density, and total root biomass among the three turfgrasses in response to supraoptimal temperatures.

Materials and Methods

The experiment was conducted twice. Laser and Pulsar RBG (0.16 g per pot, or approximately 98 kg ha^{-1}) and 'Second Millennium' TF (0.6 g per pot, or approximately 391 kg ha^{-1}) were seeded in the Throckmorton Plant Sciences Center Greenhouse Complex at Kansas State University on 30 September 2011 (run 1) and 11 November 2011 (run 2). Soil was a calcined clay (Turface MVP, Profile Products LLC, Buffalo Grove, IL) retained in 12.7 × 12.7 × 30.5 cm (length × width × height) pots. After seeding, each container received a topdressing of a controlled release fertilizer [Osmocote 14-14-14 (N-P_2O_5-K_2O), Everris NA Inc., Dublin, OH] to provide N at 24 kg ha^{-1}. Seedlings were irrigated with an automatic misting system for five minutes six times per day until 28 days after seeding. After removal from the misting system, seedlings were watered to field capacity daily, and fertilized with water-soluble fertilizer [Jack's High Performance Fertilizer 25-5-15 (N-P_2O_5-K_2O), J.R. Peters Inc., Allentown, PA] to provide N at 12 kg ha^{-1} weekly until fully covered. Grasses were clipped once weekly at 6.4 cm.

After approximately four months in the greenhouse, pots were placed into growth chambers (Conviron E15, Winnipeg, Canada). Two growth chambers were available and were randomly designated as low (23°C day/19°C night) and high (35/29°C) temperature treatments. The experiment was designed similar to Rutledge et al. (2012b), except here a split-plot, rather than a split-block, treatment structure was used in a completely randomized design. Temperature treatment (growth chamber) was the whole-plot treatment factor and turfgrass species/cultivar (TF, Pulsar, and Laser) was the sub-plot treatment factor. Three replicate sub-plots of each turf were placed in growth chambers, set at 23/19°C on 13 January 2012 (run 1). Following a four-day acclimation period, the low-temperature chamber was left at 23/19ºC while the day/night temperature was increased in the high-temperature chamber by 3/2.5°C for four days until the temperature reached 35/29°C. Turfgrasses were then subjected to high temperature treatment for 35 days. The experiment was repeated on 24 February 2012 (run 2). Plants were provided a 14-

hour photoperiod in both runs. Photosynthetically active radiation (PAR) was measured with a ceptometer (LP-80 AccuPAR PAR/LAI Ceptometer, Decagon Devices, Inc., Pullman, WA). In run 1 and run 2 PAR averaged 762 (± 54) and 781 (± 24) μmolm^{-2}s^{-1}, respectively, in a horizontal plane approximately 30 cm above the turf canopy.

Turfgrass quality, clipping yield, Pg, and EL were measured weekly. Turfgrass quality was evaluated visually considering color, density, and uniformity on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity). Clipping yield was determined by collecting clippings removed after mowing with scissors each week. Weekly yields were oven-dried at 60°C for two days and weighed. Gross photosynthesis was estimated by monitoring carbon dioxide fluxes during consecutive “sunlit” and shaded measurements with a custom steady-state chamber attached to a portable photosynthesis system (LI-6400, Li-Cor Industries, Lincoln, NE) (Bremer and Ham, 2005). Shaded measurements were obtained by covering the chamber with an opaque fabric that blocked solar radiation. Equations [5] and [6] from Bremer and Ham (2005), explain that sunlit chamber measurements estimate Pg - (Rc + Rs) and shaded chamber measurements estimate Rc + Rs, where Rc is canopy respiration and Rs is soil respiration; all values are defined as positive. Equation [8] was then used to derive Pg: Pg = (CO_2 flux from sunlit chamber) + (CO_2 flux from shaded chamber).

The EL technique used was similar to that done by Su et al. (2009). Leaf samples were collected weekly from the greenest turf in the pot. For each sample, three 2.5 cm segments were collected from fully expanded leaves and placed in a test tube containing 25 mL of distilled water. Samples were then agitated for 24 hours to remove electrolytes adhering to and released from cutting plant tissue. After shaking for 24 hours, the electrical conductivity of the solution in each test tube was measured, and test tubes were placed in a 90°C water bath for one hour. After agitating samples for an additional 24 hours, final electrical conductivity measurements were taken (% EL = initial electrical conductivity / final electrical conductivity × 100).

Shoot biomass was collected at 35 DAI and a 5 cm (diameter) × 17.5 cm (depth) plug was then randomly removed from each pot. Roots were washed, dyed with a methyl blue [acid blue 93 ($C_{37}H_{27}N_3O_9S_3Na_2$), Sigma Chemical Co., St. Louis, MO] and water solution (5 g methyl blue L^{-1} water), scanned at 600 dpi, and analyzed with WinRHIZO (version 2003 b, Regent Instruments, Quebec City, Canada) to determine root length density (RLD). Total root and shoot biomasses were then oven-dried at 60°C for two days and weighed.

Data Analysis

Residual normality was tested with the *w* statistic of the Shapiro-Wilk test using the UNIVARIATE procedure of Statistical Analysis System (SAS Institute Inc., Cary, NC) (Shapiro and Wilk, 1965). Temperature treatment × turfgrass species/cultivar treatment combinations were of most interest. For this reason, only sub-plot (turf species) and interaction effects were considered during analysis. Error variances were homogeneous between runs according to Levene's Homogeneity of Variance Test and runs were combined for analysis. All data were subjected to analysis of variance using the GLIMMIX procedure of SAS. Fisher's Protected LSD ($P \leq 0.05$) was used to detect treatment differences. Furthermore, because direct comparisons between each species/cultivar at high and low temperature were of interest, a set of pre-planned, single-degree-of-freedom contrasts (*t* tests) ($P \leq 0.05$) were used to compare each turf at high and low temperatures.

Results and Discussion

With the exception of clipping yield, root biomass, and RLD data, there were significant temperature treatment × turfgrass species/cultivar interactions for all parameters evaluated. Since treatment combinations are the most meaningful data in this experiment, effects of interactions will be presented for turfgrass quality, Pg, EL, and shoot biomass. Results of *t* tests will be presented for all parameters.

When exposed to the 23/19ºC temperature regime, Laser, Pulsar, and TF maintained acceptable quality (>6.0) for the duration of the experiment. However, at 35ºC only TF maintained acceptable quality for 35 d (Figure 2.1, Table 2.1). At 35°C, Laser and Pulsar exhibited unacceptable quality after 7 and 14 DAI, respectively, with no differences between the two thereafter. Both cultivars had an average quality rating of 2.8 by 35 DAI. In contrasts comparing each turfgrass's quality at 35 to 23ºC, quality was significantly lower at 35°C by 7 DAI for Laser and Pulsar, and by 14 DAI for TF (Table 2.1). Rough bluegrass decline was similar to that described by Rutledge et al. (2012b) who exposed Laser to 33°C for 35 d, and observed unacceptable RBG quality (< 6.0) by 28 DAI.

Gross photosynthesis of Laser, Pulsar, and TF remained relatively unchanged at 23°C, but declined over 35 d at 35°C. At 23°C, Pg of Laser and Pulsar was greater than that of TF on 7 DAI, and Pulsar also had a significantly higher Pg estimate than Laser on 28 DAI (Table 2.2).

At 35°C, TF had higher Pg estimates than Laser and Pulsar on 7 and 28 DAI. Gross photosynthesis of Pulsar was greater than that of Laser on 7 DAI, but estimates were similar after 28 days of heat stress. In contrasts comparing Pg at 35 and 23°C, Pg was significantly lower at 35°C by 0 DAI for Laser, 7 DAI for Pulsar, and 14 DAI for TF with corresponding Pg reductions of 36% for Laser and Pulsar and 26% for TF at 35°C by 35 DAI (Table 2.2).

Electrolyte leakage was never > 30% for Laser or Pulsar at 23°C, and there were no differences between the two under optimum growing conditions. Electrolyte leakage was never > 16% for TF at 23°C, and TF had significantly less EL compared to Laser and Pulsar on 21 DAI (Table 2.3). Maximum EL at 35°C was 49% for Laser, 63% for Pulsar, and 24% for TF. At 35°C, Laser and Pulsar exhibited greater EL than TF on 21 and 35 DAI. Furthermore, EL of Pulsar (62%) was significantly greater than that of Laser (46%) on 35 DAI. In contrasts comparing EL at 35 and 23°C, EL was significantly greater at 35°C by 14 DAI for Pulsar and 21 DAI for Laser. Tall fescue EL at 35°C was never greater than at 23°C. Jiang and Huang (2001) observed increased EL of relatively heat intolerant perennial ryegrass compared to relatively heat tolerant TF when the species were exposed to heat stress. Electrolyte leakage estimates cell membrane thermostability, and has been used to predict whole-plant heat tolerance among Kentucky bluegrass cultivars (Marcum, 1998). While TF heat tolerance is associated with its deep rooting characteristics (Fry and Huang, 2004), reduced EL from TF compared to RBG cultivars in this study emphasizes the importance of the maintenance of cell membrane function during heat stress. Su et al. (2009) observed differing membrane lipid compositions and greater saturation of fatty acids in heat-tolerant compared to heat-sensitive cool-season turfgrassess. Similar trends between TF and RBG are likely. Furthermore, the increased EL in Pulsar could explain the relative heat tolerance of Laser compared to Pulsar observed by Morton et al. (2009), but Pg of Laser was affected by heat stress sooner than Pulsar in this study. It is also important to reiterate that Rutledge et al. (2012b) did not observed differences in EL between Laser and Pulsar.

There was never a significant interaction between turfgrass clipping yields and temperature treatments (Table 2.4). At 35°C, clipping yields on 7 DAI were 3.7 mg cm^{-2} for Laser, 5.0 mg cm^{-2} for Pulsar, and 5.6 mg cm^{-2} for TF, significantly less than produced by each turf at 23°C according to contrasts. This trend continued throughout the 35 d treatment period, and on 35 DAI clipping yields of turfgrasses at 35°C were 0.0 mg cm^{-2} for Laser, 0.6 mg cm^{-2} for

Pulsar, and 1.9 mg cm^{-2} for TF. Similar to observations by Rutledge et al. (2012b), Laser subjected to 35°C had ceased growing by 28 DAI.

At 35 DAI, TF grown at 23°C had accumulated more shoot biomass (68.2 mg cm^{-2}) than any other turf × temperature treatment combination, and shoot biomass accumulated by TF at 35°C (62.0 mg cm^{-2}) was not different from Laser and Pulsar at 23°C (62.0 and 55.8 mg cm^{-2}, respectively) (Table 2.5). Laser and Pulsar both accumulated 31.0 mg cm^{-2} of shoot biomass at 35°C, less than any other turf × temperature treatment combination. According to contrasts, all turfgrasses had less shoot biomass at 35°C compared to 23°C.

Concerning root biomass and RLD, there was not a significant turf × temperature treatment interaction on 35 DAI. However, contrasts revealed that root biomass in the top 17.5 cm of pots and RLD of Laser and Pulsar at 35°C was significantly less than that at 23°C (Table 2.5). Accumulated root biomass in the top 17.5 cm of pots and RLD of TF was similar at 35 and 23°C. Decreasing soil temperatures for root health maintenance while maintaining 35°C air temperatures have been shown to increase turf quality, tiller density, leaf chlorophyll content, and shoot growth rate of creeping bentgrass, indicating that root heath maintenance is critical for plant survival (Xu and Huang, 2000). The significant decline in root biomass and RLD of Laser and Pulsar from 23 to 35°C likely contributed to reduced turf quality (Table 2.1) and Pg (Table 2.2) of RBG cultivars at 35°C. Still, the lack of rooting differences between Laser and Pulsar at 35°C with significant differences in EL between the two (Table 2.3) indicate that differences in cell membrane thermostability could play a significant role in heat tolerance among RBG cultivars.

Conclusions

The superior heat tolerance of TF compared to RBG is associated with maintenance of rooting, photosynthesis, and cell membrane viability at elevated temperatures. Few differences were observed between Laser and Pulsar RBG subjected to heat stress. Both cultivars exhibited a similar level of poor quality, decreased photosynthetic activity, reduced clipping yields, and reduced rooting at elevated temperatures. Pulsar has been regarded as more heat-sensitive than Laser. While we didn’t observe differences in decline between the two cultivars, Laser did exhibit a higher degree of cell membrane thermostability compared to Pulsar, which may contribute to the relative heat-tolerance of Laser.

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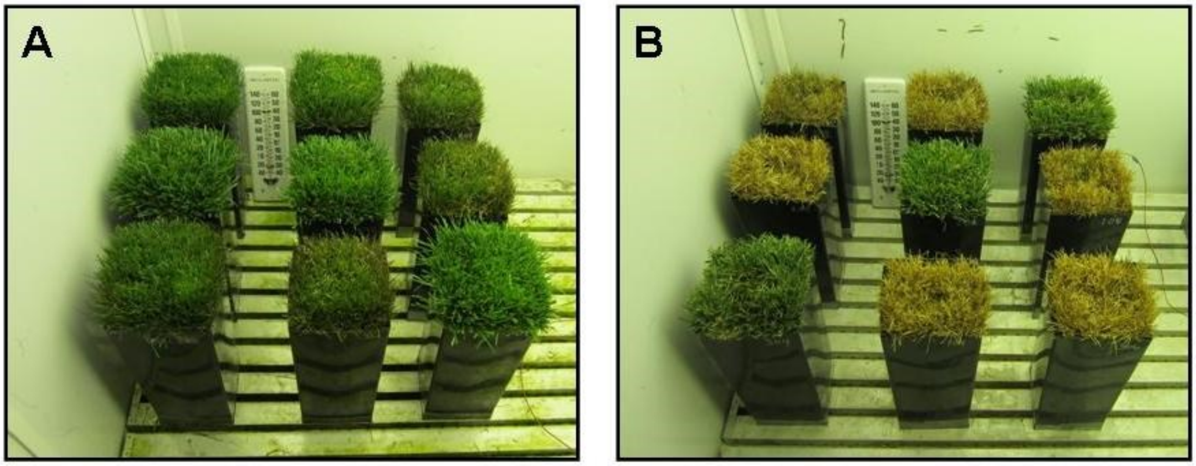

Figure 2.1 Effects of optimal and supraoptimal temperatures on the quality of rough bluegrass cultivars and tall fescue 35 days after the induction of heat stress in controlled environment chambers. A) All turfgrasses have acceptable quality at optimal (23°C day/19°C night) temperatures. B) Only tall fescue has acceptable quality at supraoptimal (35°C day/29°C night) temperatures.

Table 2.1 Effects of optimal and supraoptimal temperature on the quality of rough bluegrass cultivars and tall fescue grown in controlled environment chambers.

	Quality[†]					
	0 DAI[‡]	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
(23/19°C)						
Laser	7.8	8.2 a[§]	8.2 a	7.8 b	7.7 b	7.2 b
Pulsar	8.3	8.0 a	8.3 a	8.0 b	8.0 b	7.5 b
Tall fescue	8.3	8.3 a	8.5 a	8.7 a	8.7 a	8.3 a
(35/29°C)						
Laser	7.7	5.7 c	4.8 c	4.3 d	3.5 d	2.8 c
Pulsar	8.2	6.5 b	4.8 c	4.3 d	3.2 d	2.8 c
Tall fescue	8.7	7.8 a	7.5 b	7.0 c	7.0 c	7.0 b
Contrasts (23/19°C vs. 35/29°C)[¶]						
Laser	NS	***	***	***	***	***
Pulsar	NS	***	***	***	***	***
Tall fescue	NS	NS	**	***	***	***

[†] Turfgrass quality was rated visually considering color, density, and uniformity on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity).

[‡] Days after induction (DAI) of heat treatment.

[§] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[¶] A set of single-degree-of-freedom contrasts was used to compare Laser, Pulsar, and tall fescue at optimal and supraoptimal temperatures.

*, **, and *** are significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 2.2 Effects of optimal and supraoptimal temperature on gross photosynthesis (Pg) of rough bluegrass cultivars and tall fescue grown in controlled environment chambers.

	Pg (μmol CO_2 m^{-2} s^{-1})[†]					
	0 DAI[‡]	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
(23/19°C)						
Laser	15	16 a[§]	17	14	13 b	12
Pulsar	15	16 a	18	15	16 a	12
Tall fescue	15	13 bc	19	17	15 ab	15
(35/29°C)						
Laser	12	8 d	9	7	5 d	4
Pulsar	16	12 c	7	6	5 d	4
Tall fescue	15	15 ab	14	11	11 c	11
Contrasts (23/19°C vs. 35/29°C)[¶]						
Laser	*	***	***	***	***	***
Pulsar	NS	**	***	***	***	***
Tall fescue	NS	NS	**	***	***	***

[†] Gross photosynthesis was estimated from the sum of sunlit and shaded measurements taken with a portable photosynthesis chamber.

[‡] Days after induction (DAI) of heat treatment.

[§] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD (*P* ≤ 0.05).

[¶] A set of single-degree-of-freedom contrasts was used to compare Laser, Pulsar, and tall fescue at optimal and supraoptimal temperatures.

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*, **, and *** are significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 2.3 Effects of optimal and supraoptimal temperature on electrolyte leakage (EL) of rough bluegrass cultivars and tall fescue grown in controlled environment chambers.

	Electrolyte Leakage (%)[†]					
	0 DAI[‡]	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
(23/19°C)						
Laser	23	29	28	32 b[§]	27	24 c
Pulsar	24	30	23	24 bc	29	22 c
Tall fescue	13	16	15	12 d	12	12 c
(35/29°C)						
Laser	23	37	37	45 a	49	46 b
Pulsar	25	27	40	57 a	63	62 a
Tall fescue	13	18	16	18 cd	24	20 c
	Contrasts (23/19°C vs. 35/29°C)[¶]					
Laser	NS	NS	NS	*	**	***
Pulsar	NS	NS	*	***	***	***
Tall fescue	NS	NS	NS	NS	NS	NS

[†] Leaf segments were agitated for 24 hours to remove electrolytes adhering to, and released from severed plant tissue. After shaking for 24 hours, the electrical conductivity of the solution was measured, and test tubes were placed in a 90°C water bath for one hour. After agitating samples for an additional 24 hours, final electrical conductivity measurements were taken (% EL = initial electrical conductivity / final electrical conductivity × 100).

[‡] Days after induction (DAI) of heat treatment.

[§] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[¶] A set of single-degree-of-freedom contrasts was used to compare Laser, Pulsar, and tall fescue at optimal and supraoptimal temperatures.

*, **, and *** are significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 2.4 Effects of optimal and supraoptimal temperature on clipping yields of rough bluegrass cultivars and tall fescue grown in controlled environment chambers.

	Clipping yield (mg cm^{-2})[†]					
	0 DAI[‡]	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
(23/19°C)						
Laser	0.0	6.8	7.4	8.1	5.0	3.7
Pulsar	0.0	8.7	8.7	10.5	6.8	5.0
Tall fescue	0.0	9.9	9.9	13.0	9.3	7.4
(35/29°C)						
Laser	0.0	3.7	1.2	1.2	0.0	0.0
Pulsar	0.0	5.0	1.9	1.9	0.6	0.6
Tall fescue	0.0	5.6	3.7	3.7	2.5	1.9
	Contrasts (23/19°C vs. 35/29°C)[§]					
Laser	NS	*	***	***	***	***
Pulsar	NS	**	***	***	***	***
Tall fescue	NS	***	***	***	***	***

[†] Clipping yields were determined by collecting clippings produced weekly. Clippings were oven-dried at 60°C for two days, and then weighed.

[‡] Days after induction (DAI) of heat treatment.

[§] A set of single-degree-of-freedom contrasts was used to compare Laser, Pulsar, and tall fescue at optimal and supraoptimal temperatures.

*, **, and *** are significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 2.5 Effects of optimal and supraoptimal temperature on shoot biomass, root biomass, and root length density (RLD) of rough bluegrass cultivars and tall fescue grown in controlled environment chambers.

	Shoot biomass[†]	Root biomass[‡]	RLD[§]
	---------------------mg cm^{-2}---------------------		$cm\ cm^{-3}$
(23/19°C)			
Laser	62.0 b[¶]	11.2	34
Pulsar	55.8 b	15.3	40
Tall fescue	68.2 a	11.7	19
(35/29°C)			
Laser	31.0 c	8.7	24
Pulsar	31.0 c	11.2	37
Tall fescue	62.0 b	10.7	16
	Contrasts (23/19°C vs. 35/29°C)[#]		
Laser	***	*	**
Pulsar	***	**	*
Tall fescue	**	NS	NS

[†] Total shoot biomass was collected at 35 DAI, oven-dried at 60°C for two days, and weighed.

[‡] At 35 DAI, a 5 cm (diameter) × 17.5 cm (depth) plug was randomly removed from each pot. Roots were washed, oven-dried at 60°C for two days, and weighed.

[§] Root length density (RLD) was determined by analyzing clean roots from a 5 cm (diameter) × 17.5 cm (depth) plug with WinRHIZO.

[¶] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[#] A set of single-degree-of-freedom contrasts was used to compare Laser, Pulsar, and tall fescue at optimal and supraoptimal temperatures.

*, **, and *** are significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Chapter 3 - Physiological and Pathological Contributors to Summer Rough Bluegrass Decline

Abstract

Rough bluegrass (RBG, *Poa trivialis* L.) commonly develops as a weed in cool-season turfgrass swards after its unintended presence as a contaminant in seed lots of desirable grasses. Rough bluegrass often declines during mid-summer due to biotic or abiotic stresses. The overall goal of this research was to differentiate between physiological and pathological contributors to RBG decline, and determine the effects of QoI fungicides on growth and physiological parameters in controlled environment and field studies. Rough bluegrass was treated with azoxystrobin (Heritage 50 WDG or Heritage TL) at 610 g a.i. ha^{-1} or pyraclostrobin (Insignia 20 WG or Insignia SC) at 556 g a.i. ha^{-1} and exposed to heat stress (35°C day/29°C night) in growth chambers and in the field in Manhattan, KS (2011 and 2012) and Mead, NE (2012). Fungicide treatments had no effect on RBG quality, gross photosynthesis (Pg), clipping yield, electrolyte leakage (EL), shoot biomass, root biomass, or root length density (RLD) in growth chambers. In field studies, fungicide treatments generally did not influence EL, root biomass, or RLD of RBG. However, fungicide treatments did improve RBG quality, cover, and Pg when decline was observed during summer, and turf treated with azoxystrobin had higher measurements for all aforementioned parameters compared to untreated turf. The increased Pg of fungicide-treated RBG compared to untreated RBG during stress likely resulted from delayed leaf senescence (improved quality and cover). Evaluation of RBG foliage and roots did not reveal a fungal pathogen consistently associated with RBG decline. Physiological stresses are likely the primary cause of summer RBG decline. It is unclear why QoI fungicides positively affect heat-stressed RBG in the field, but poorly understood non-target effects may be the cause.

Introduction

Rough bluegrass (RBG, *Poa trivialis* L.) is a cool-season perennial turfgrass species that often declines during summer due to abiotic and/or biotic stresses (Beard, 1973). Many commonly used cool-season turfgrass species are less sensitive to heat stress than RBG (Sifers and Beard, 1993). High temperature stress is the main factor causing leaf senescence and physiological damage of cool-season turfgrasses (Cross et al., 2013; Xu and Huang, 2009) and occurs at temperatures greater than 30°C (Fry and Huang, 2004). Photosynthetic rates are more inhibited by high temperature stress than respiratory rates resulting in an imbalance whereby carbon used by respiration exceeds that provided by photosynthesis, ultimately depleting carbohydrate reserves (Taiz and Zeiger, 2010). The reduced photosynthetic capacity of plants exposed to heat stress has been associated with reductions in photochemical (e.g. carotenoids, chlorophyll a and b) efficiency of photosystem II, the interruption of electron transport, and reduced CO_2 fixation and assimilation resulting from reduced ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) activity (Berry and Björkman, 1980; Liu and Huang, 2008, Xu and Huang, 2000). Additionally, the induction of free radicals such as hydrogen peroxide (H_2O_2) during heat stress results in lipid peroxidation, ultimately degrading cell membranes and possibly inhibiting photosynthesis and respiration (Fry and Huang, 2004).

While the increased sensitivity of RBG to high temperature stress compared to other turfgrass species is often associated with increased respiration and/or decreased photosynthesis (Carroll and Welton, 1937; Loveys et al., 2002; Watschke et al., 1973), Rutledge et al. (2012b) recently observed differences in creeping bentgrass (*Agrostis stolonifera* L.) and RBG shoot amino acid concentrations and root nonstructural carbohydrate concentrations during heat stress. After 35 days at 33°C, RBG shoot amino acid concentrations increased 223% compared to plants at 23°C. Rough bluegrass roots also maintained higher total nonstructural carbohydrate and fructan concentrations than creeping bentgrass roots, indicating that RBG may not have been able to hydrolyze fructan to simple sugars for metabolic activity (Rutledge et al., 2012b). In a separate study, Rutledge et al. (2012a) observed that total nonstructural carbohydrate concentrations in ‘Laser’ and ‘Pulsar’ RBG shoots in midsummer decreased 18 to 26%, possibly due to degradation, metabolism, or translocation to stolons. In fall, the researchers observed an influx of nonstructural carbohydrates to shoots, presumably from stolons, suggesting that the rapid decline of RBG during high temperature stress is a survival mechanism whereby stolons are used as survival structures until favorable growing conditions return.

Rough bluegrass summer decline may also be exacerbated by its susceptibility to several common turfgrass diseases (Hurley, 2003). Golf course superintendents often anecdotally report earlier and more severe incidences of dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) on RBG compared to desirable species. Furthermore, RBG subjected to repeated applications of fungicide mixtures containing azoxystrobin maintained quality and cover during summer months in Indiana compared to RBG not treated with azoxystrobin (Weisenberger and Reicher, 2006 and 2007), indicating that summer RBG decline may be due, in part, to one or more pathogens. In fungi, the strobilurin (QoI) fungicides (Fungicide Resistance Action Committee [FRAC] Code 11) prevent ATP synthesis by inhibiting mitochondrial respiration at the Q_o site of cytochrome *b*, blocking electron transfer between cytochrome *b* and c_1 (Bartlett et al., 2002). The QoI fungicides offer broad-spectrum disease control, but have also been recently associated with plant heath and productivity not related to disease control. Researchers have shown that treatment with azoxystrobin, kresoxim-methyl, trifloxystrobin, picoxystrobin, or pyraclostrobin have positively affected yield and grain size in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) even with insignificant differences in disease control compared to other fungicide groups (Bartlett et al., 2002). One theory explaining this phenomenon includes non-target, physiological effects of fungicidal compounds in host plants. Applications of kresoxim-methyl or pyraclostrobin to wheat under drought stress evoked similar plant responses to low quantities of auxins reducing ethylene activity and increasing cytokinin content and antioxidant activity (Grossmann and Retzlaff, 1997; Köhle el al., 2002). More recently, researchers in Tennessee examined the effects of QoI fungicides on creeping bentgrass under heat and/or drought stress (Brosnan et al., 2010). No changes in turfgrass quality were observed after fungicide applications, but azoxystrobin reduced visual root length and total root biomass of ‘Penncross’ creeping bentgrass, and total root length, root length density, and total root biomass of ‘Penn A-1’ creeping bentgrass compared to untreated turf at 27°C under well-watered conditions. Conversely, treatment with pyraclostrobin increased visual root length for both cultivars and also increased total root length, root surface area, root length density, root volume, and root biomass for Penn A-1 compared to untreated turf at 27°C and irrigated to prevent leaf wilt.

Considering previous physiological evidence (Carroll and Welton, 1937; Loveys et al., 2002; Rutledge et al., 2012a; Rutledge et al., 2012b; Sifers and Beard, 1993; Watschke et al., 1973), it is unclear why QoI fungicides might prevent severe RBG summer decline. Fungicidal compounds could be simply protecting RBG from a fungal pathogen, or possibly having non-target physiological effects on RBG. Therefore, the objectives were to: 1) differentiate between physiological and potential pathological contributors to summer RBG decline and 2) observe the effects of QoI fungicides on summer RBG decline.

Materials and Methods

Growth Chamber Study

Laser RBG was established in the Throckmorton Plant Sciences Center Greenhouse Complex at Kansas State University on 23 December 2011 (replicates 1 and 2) and 3 February 2012 (replicates 3 and 4). Rough bluegrass was seeded at 98 kg ha^{-1} in 12.7 × 12.7 × 30.5 cm (length × width × height) pots filled with calcined clay (Turface MVP, Profile Products LLC, Buffalo Grove, IL) and topdressed with 24 kg N ha^{-1} of controlled release fertilizer [Osmocote 14-14-14 (N-P_2O_5-K_2O), Everris NA Inc., Dublin, OH]. Seedlings were irrigated with an automatic mist system for five minutes six times per day until 28 days after seeding. After removal from the mist system, seedlings were watered to field capacity daily, and fertilized with water-soluble fertilizer [Jack's High Performance Fertilizer 25-5-15 (N-P_2O_5-K_2O), J.R. Peters Inc., Allentown, PA] at 12 kg N ha^{-1} weekly until fully covered. Grasses were clipped once weekly at 6.4 cm.

After approximately three months in the greenhouse, pots were placed into growth chambers (Conviron E15, Winnipeg, Canada) set at 23/19°C (day/night) on 1 June 2012 (replicates 1 and 2) and 13 July 2012 (replicates 3 and 4). Following a four-day acclimation period, temperatures were increased by 3/2.5°C for four days until temperatures reached 35/29°C. Pots of RBG were subjected to high temperature treatment for 35 days. The experiment was conducted once as randomized complete-block design with four replications (blocks). Only two growth chambers were available, and each served as a separate block on two different timings to achieve the experimental design. In each replicate block there was one pot of RBG for each fungicide treatment. Plants were provided a 14 hour photoperiod. Photosynthetically active radiation (PAR) was measured with a ceptometer (LP-80 AccuPAR

PAR/LAI Ceptometer, Decagon Devices, Inc., Pullman, WA) and averaged 750 (±27) $\mu molm^{-2}s^{-1}$ in a horizontal plane approximately 30 cm above the turf canopy. Fungicide treatments were applied 7 days prior to heat treatment (-7 days of treatment), and at 7 and 21 days after induction of heat treatment (DAI) to evaluate stress mitigation potential. Treatments included an untreated control, two formulations of azoxystrobin {methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin -4-yloxy]phenyl}-3 methoxyacrylate; Heritage 50 WDG and Heritage TL, Syngenta Crop Protection, Greensboro, NC} at 610 g a.i. ha^{-1}, and two formulations of pyraclostrobin {(carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl] phenyl]methoxy-,methyl ester); Insignia 20 WG and Insignia SC, BASF Corporation, Research Triangle Park, NC} at 556 g a.i. ha^{-1}. Two formulations of each active ingredient were used because Brosnan et al. (2010) attributed the differing effects of azoxystrobin and pyraclostrobin on creeping bentgrass root development in their study to differences in solubility and phytomobility between fungicidal compounds, and recommended further research with different formulations. Applications were made with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa.

Turfgrass quality, clipping yield, gross photosynthesis (Pg), and electrolyte leakage (EL) were measured weekly. Turfgrass quality was taken considering color, density, and uniformity on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity). Clipping yields were estimated by collecting clippings produced weekly. Clippings were oven-dried at 60°C for two days, and then weighed. Gross photosynthesis was estimated by monitoring carbon dioxide fluxes during consecutive "sunlit" and shaded measurements with a custom steady state chamber attached to a portable photosynthesis system (LI-6400, Li-Cor Industries, Lincoln, NE) (Bremer and Ham, 2005). Shaded measurements were obtained by covering the chamber with an opaque fabric that blocked solar radiation. Equations [5] and [6] from Bremer and Ham (2005), explain that sunlit chamber measurements estimate Pg - (Rc + Rs) and shaded chamber measurements estimate Rc + Rs, where Rc is canopy respiration and Rs is soil respiration; all values are defined as positive. Equation [8] was then used to derive Pg: Pg = (CO_2 flux from sunlit chamber) + (CO_2 flux from shaded chamber).

Electrolyte leakage is a measure of cell membrane thermostability that is commonly used to evaluate relative heat tolerance of plants (Jiang and Huang, 2001; Marcum, 1998; Su et al., 2009). The EL technique used was similar to that done by Su et al. (2009). Leaf samples were collected weekly from the greenest areas in each pot. For each sample, three 2.5 cm segments were collected from fully expanded leaves and placed in a test tube containing 25 mL of distilled water. Samples were then agitated for 24 hours to remove electrolytes adhering to and released from severed plant tissue. After shaking for 24 hours, the electrical conductivity of the solution in each test tube was measured, and test tubes were placed in a 90°C water bath for one hour. After agitating samples for an additional 24 hours, final electrical conductivity measurements were taken (% EL = initial electrical conductivity / final electrical conductivity × 100).

Shoot biomass was collected at 35 DAI and a 5 cm (diameter) × 17.5 cm (depth) plug was then randomly removed from each pot. Roots were washed, died with a methyl blue [acid blue 93 ($C_{37}H_{27}N_3O_9S_3Na_2$), Sigma Chemical Co., St. Louis, MO] and water solution (5 g methyl blue L^{-1} water), scanned at 600 dpi, and analyzed with WinRHIZO (version 2003 b, Regent Instruments, Quebec City, Canada) to determine root length density (RLD), surface area, and average diameter. Root and shoot biomasses were then oven-dried at 60°C for two days and weighed.

Field Studies

Studies were conducted at the Rocky Ford Turfgrass Research Center in Manhattan, KS in 2011 and 2012 and at the John Seaton Anderson Turf Research Center in Mead, NE in 2012. Research plots (0.9 × 0.9 m in Manhattan and 1.5 × 1.5 m in Mead) were arranged in a randomized complete-block design with four replications. In Manhattan, the study was conducted on Laser RBG originally seeded in the fall of 2009. Soil was a Chase silt loam (fine, smectitic, mesic, Aquertic Argiudoll) with a pH of 7.6 and phosphorous and potassium levels of 0.11 and 0.42 g kg^{-1}, respectively. In Mead, the study was conducted on 'Winterstar' RBG originally seeded in the fall of 2010. Soil was a Tomek silty clay loam (fine, montmorillonitic, mesic, Typic Argiudoll) with a pH of 7.5 and phosphorous and potassium levels of 0.03 and 0.50 g kg^{-1}, respectively. Research areas were irrigated as needed to prevent drought stress and mowed at 6.3 cm once weekly with a rotary mower.

In Manhattan, N was applied at 49 kg ha^{-1} on 18 March, 2 May, 19 September, and 10 November 2011 and on 26 March, 15 May, 18 September, and 9 November 2012 to provide a total of 195 kg ha^{-1} annually. Polymer-coated urea (41-0-0 N-P_2O_5-K_2O; Polyon/Pursell

Industries, Sylacauga, AL) was used on 2 May 2011 and 15 May 2012, and urea (46-0-0) was used on all other dates. Dimension 2 EW [dithiopyr: S,S'-dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioate; Dow AgroSciences LLC, Indianapolis, IN] and Speed Zone {Carfentrazone-ethyl: Ethyl α,2-dichloro-5-[4(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate; 2,4-D, 2-ethylhexyl ester: 2,4-dichlorophenoxyacetic acid equivalent; Mecoprop-p acid: (+)-R-2-(2-methyl-4-chlorophenoxy)propionic acid equivalent; and Dicamba acid: 3,6-dichloro-o-anisic acid equivalent; PBI/Gordon Corporation, Kansas City, MO} were applied at 0.6 kg a.i. ha^{-1} and 1.3 kg a.i. ha^{-1}, respectively, on 12 April 2011 and 26 March 2012 for common dandelion (*Taraxacum officinale* Wigg.) control and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Muhl.] and large crabgrass [*D. sanguinalis* (L.) Scop.] prevention. Additionally, Merit 0.5 G {Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine; Bayer Environmental Science, Research Triangle Park, NC} was applied at 0.3 kg a.i. ha^{-1} on 14 April 2011 and 29 May 2012 for control of southern masked chafer (*Cyclocephala lurida* Bland) and May beetle (*Phyllophaga* spp.) larvae.

In Mead, polymer-coated urea was used to provide N at 49 kg ha^{-1} on 1 May, 1 September, and 1 November 2012 for a total of 147 kg ha^{-1} annually. Pendimethalin (N-[1-ethylpropyl]-3, 4-dimethyl-2, 6-dinitrobenzenamine) was applied in late-April 2012 at 3.4 kg a.i. ha^{-1} and Trimec Classic (Dimethylamine salt of 2,4-dichlorophenoxyacetic acid; dimethylamine salt of [+]-[R]-2-[2-methyl-4-chlorophenoxy]propionic acid; and dimethylamine salt of dicamba: 3,6-dichloro-o-anisic acid; PBI/Gordon Corporation, Kansas City, MO) was applied at 1.5 kg a.i. ha^{-1} in late-September 2012 for broadleaf weed control.

Heritage 50 WDG, Heritage TL, Insignia 20 WG, and Insignia SC were applied as described for the growth chamber study on a two-week interval from 21 May to 23 August in Manhattan in 2011. In 2012, fungicides were applied from 23 April to 30 August in Manhattan and from 4 May to 30 August in Mead.

In Manhattan (2011), Manhattan (2012), and Mead (2012) turfgrass quality and percentage green turf coverage were visually estimated every month from 31 May to 11 November 2011, 30 May to 13 November 2012, and 18 May to 28 September 2012, respectively, and percentage green RBG cover was also collected on 30 May 2012, 24 May 2013, and 3 June 2013, respectively. Electrolyte leakage samples were collected weekly from the greenest turf in each plot in Manhattan from 25 May to 31 August 2011 and from 6 June to 30 August 2012. Gross photosynthesis measurements were taken weekly in the center of each plot in Manhattan from 31 May to 8 September 2011 and from 4 June to 6 September 2012. In Mead, Pg measurements were taken monthly from 28 June to 26 September 2012. Turfgrass quality was recorded as described for the growth chamber study and percent green cover data were taken as a visual estimate of each plot covered by RBG. Gross photosynthesis was measured as described for the growth chamber study, except carbon dioxide fluxes were monitored with a non-steady state chamber that was developed at Kansas State University and configured with a closed path infrared gas analyzer (LI-840, Li-Cor Industries, Lincoln, NE) (Lewis, 2010). In Manhattan and Mead in 2012, 5 cm (diameter) × 17.5 cm (depth) plugs were removed from each plot on 24 and 28 August, respectively, then washed, died with methyl blue, and analyzed with WinRHIZO to determine RLD, surface area, and average root diameter. After analysis, roots were dried at 60°C for 2 days and then weighed to determine root biomass.

In Manhattan, plots were continuously monitored for signs and symptoms of foliar diseases from 31 May to 11 November 2011 and from 30 May to 13 November 2012. Plots were also sampled for the presence of pathogens when RBG appeared healthy on 24 May 2011 and 9 June 2012 and when RBG had declined on 11 July 2011 and 7 August 2012. On 24 May 2011, two 2.5 cm (diameter) × 15.0 cm (depth) plugs were removed from each plot and incubated in a sealed, clear bag with a moist paper towel. Foliage was inspected for lesions, mycelia growth, and other symptoms and signs the following day, and roots were soaked in water overnight to loosen field-soil. Soil was washed from roots the following day, and roots were examined microscopically for the presence of pathogens and overall health. On 11 July 2011, 9 June 2012, and 7 August 2012 one 10.2 cm (diameter) × 15.0 cm (depth) plug was removed from each plot. Plugs were incubated overnight, and foliage was examined the following day. Five pieces of leaf tissue exhibiting both healthy and necrotic tissue approximately 5 mm in length were plated on one-quarter strength potato dextrose agar (¼ PDA + +). Tissue was surface sterilized in 10% sodium hypochlorite (NaOCl), rinsed in sterile water, and blotted dry before plating. Cultures were examined after three days. For root analysis, approximately 2.5 cm of the margin of each plug was removed, soaked, and soil was washed from roots. On each sampling date, roots were examined under a compound microscope in at least 10 fields of view. Roots were rated on a 1 to 5 scale (1=completely dark/discolored; 2=mostly dark/discolored; 3=approximately 50% healthy, 50% discolored, minor incidence of ectotrophic fungi; 4=minor discoloration; and 5=healthy root system). In Mead, one 5 cm (diameter) × 17.5 cm (depth) plug was removed from each untreated plot on 28 June and 27 July 2012. Samples were washed and foliage and roots were analyzed as previously described.

Data Analysis

Residual normality was tested with the *w* statistic of the Shapiro-Wilk test using the UNIVARIATE procedure of Statistical Analysis System (SAS Institute Inc., Cary, NC) (Shapiro and Wilk, 1965). Rough bluegrass cover data were not normally distributed, and were subjected to an arcsin ($y/100$) transformation prior to analysis and back-transformed for presentation. All data were subjected to analysis of variance using the GLIMMIX procedure of SAS. Because direct comparisons between each fungicide treatment and the untreated were of most interest, a set of pre-planned, single-degree-of-freedom contrasts (*t* tests) ($P < 0.05$) were used to compare fungicide treatments to the untreated control.

Results and Discussion

Growth Chamber Study

No treatment resulted in acceptable turfgrass quality for the duration of the experiment (Table 3.1). Similar to Rutledge et al. (2012b) who exposed Laser RBG to 33°C for 35 d, RBG quality was less than acceptable (< 6.0) for all treatments on 28 and 35 DAI. In this study, turfgrass quality in a controlled environment was not improved by fungicide applications, similar to previous research (Brosnan et al., 2010). Gross photosynthesis of RBG declined over the 35 day treatment period, regardless of fungicide treatment (Table 3.2). Rough bluegrass treated with Insignia 20 WG averaged greater Pg (4.8 μmol CO_2 m^{-2} s^{-1}) than untreated RBG (3.2 μmol CO_2 m^{-2} s^{-1}) on 21 DAI. No other differences were detected. There were never differences in RBG clipping yields among treatments (Table 3.3). Untreated RBG and RBG treated with QoI fungicides had clipping yields ranging from 0.9 to 1.2 mg cm^{-2} dry weight at 7 DAI. Clipping yields declined weekly and no clippings were produced by 35 DAI, regardless of fungicide treatment, which was similar to results observed when RBG was exposed to 33°C in the absence of fungicides in previous growth chamber research (Rutledge et al., 2012b). Electrolyte leakage was never greater than 33% for any treatment, EL did not increase with time of exposure to heat stress, and there were no differences among treatments (Table 3.4). Cross et al. (2013) observed 93.7% EL of a "summer stress-sensitive" tall fescue (*Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.) genotype exhibiting low quality (2.0 on a 1 to 9 scale) after four weeks of heat stress (38°C day/33°C night). Even though no differences in EL among treatments were observed in this study, EL should have at least increased with decreasing RBG quality from 0 to 35 DAI. Lack of increasing EL in this study is likely an artifact of the sampling method. Because the greenest leaf tissue was always selected during sampling, EL estimates were likely artificially depressed, and not representative of pots.

At 35 DAI, there were no differences in shoot biomass, root biomass, or RLD among treatments (Table 3.5).

Field Studies

In general, fungicide treatments did not affect RBG quality, green cover, or Pg until decline began in summer. All fungicide treatments had greater quality and green RBG cover compared to untreated RBG periodically throughout the three studies.

Rough Bluegrass Quality

In Manhattan (2011), RBG had acceptable quality in May and June, regardless of treatment (Table 3.6). By 27 July, RBG quality was unacceptable (<6.0) for all treatments and only RBG treated with Heritage TL averaged greater turf quality (5.0) than untreated RBG (2.5). On 25 August, RBG quality was < 4.0 for all treatments and all fungicide treatments resulted in greater RBG quality than untreated (1.3). Rough bluegrass quality remained unacceptable through November for all treatments, but RBG treated with Heritage 50 WDG or Heritage TL averaged greater quality compared to untreated RBG on each of the three remaining dates in 2011.

Rough bluegrass decline was not as severe in 2012 as in 2011. In Manhattan (2012), untreated RBG had unacceptable quality on only one of seven rating dates (30 August). All fungicide-treated RBG had quality > 7.0 on this date, and was significantly greater than untreated RBG (Table 3.7). By 26 September, untreated RBG was again acceptable (6.5), and all fungicide-treated RBG had quality > 8.0, significantly higher than untreated RBG. Untreated RBG quality was 7.0 or greater in October and November. In Mead in 2012, untreated RBG had unacceptable quality on three of five rating dates, but quality was never lower than 4.8 (Table

3.7). Rough bluegrass treated with Heritage 50 WDG, Heritage TL, or Insignia SC never had unacceptable quality, and that treated with Insignia 20 WG was only unacceptable (5.8) on 31 July.

Similar to the results of Weisenberger and Reicher (2007), RBG treated with QoI fungicides maintained acceptable quality during summer months (in 2012) when untreated turf did not. The summer quality of azoxystrobin-treated RBG was most consistently improved compared to untreated RBG in this study. Brosnan et al. (2010) did not observe improved quality of azoxystrobin- or pyraclostrobin-treated creeping bentgrass exposed to heat stress in a greenhouse, possibly due to the higher heat tolerance of creeping bentgrass compared to RBG (Sifers and Beard, 1993).

Rough Bluegrass Green Cover

In Manhattan (2011), all treatments averaged nearly 100% green RBG cover on 31 May and 28 June, but no treatment had > 78% green RBG cover by 27 July (Table 3.8). Untreated RBG and RBG treated with Insignia SC or Insignia 20 WG continued loss of green cover, averaging only 0.8, 8.3, and 7.5% green RBG cover, respectively, by 28 August. Rough bluegrass treated with Heritage 50 WDG or Heritage TL averaged 16.8 and 21.3% green RBG cover, respectively, at this time and both were significantly greater than untreated. All treatments continued to decline into September, but RBG treated with Heritage 50 WDG or Heritage TL maintained greater green RBG cover compared to untreated RBG through November. On 12 May 2012, RBG treated with Heritage 50 WDG, Heritage TL, or Insignia SC in 2011 averaged 87.8, 91.0, and 63.8% green RBG cover, respectively, significantly higher than untreated RBG (30.0%). Fungicides and the respective rating dates (out of 8) on which they provided more green RBG cover than untreated turf were: Heritage 50 WDG (4), Heritage TL (5), Insignia 20 WG (0), and Insignia SC (1).

Rough bluegrass decline was not as severe in 2012 as in 2011. In Manhattan (2012), untreated RBG never fell below 73.8% green cover, and fungicide treatments never had < 85.0% cover (Figure 3.1, Table 3.9). Fungicides and the respective rating dates (out of 8) on which they provided more green RBG cover than untreated turf were: Heritage 50 WDG (4), Heritage TL (5), Insignia 20 WG (5), and Insignia SC (3).

All treatments in Mead in 2012 averaged approximately 80.0% green RBG cover on 18 May (Table 3.9). Untreated RBG declined to 58.8% green RBG cover by 31 July, but recovered to 75.0% green RBG cover by 5 November, and 92.5% green RBG cover by 3 June 2013. Fungicide-treated RBG never averaged less than 70.0% green RBG cover and fungicides and the respective rating dates (out of 8) on which they provided more green RBG cover than untreated turf were: Heritage 50 WDG (7), Heritage TL (7), Insignia 20 WG (4), and Insignia SC (3).

Compared to the more modest results in Manhattan (2011), the less severe RBG decline in Manhattan and Mead in 2012 resulted in the maintenance of green RBG cover similar to that observed by Weisenberger and Reicher (2006).

Gross Photosynthesis

Rough bluegrass Pg was affected by fungicide treatments in the field studies in Manhattan and Mead, but to a lesser extent than turfgrass quality and green RBG cover. Furthermore, estimated Pg was only consistently different from untreated turf during July and August in Manhattan, when green RBG cover was significantly less than fungicide-treated RBG (Tables 3.9 and 3.10). In Manhattan (2011), Pg of unstressed RBG on 31 May ranged from 11.5 to 13.2 μmol CO_2 m^{-2} s^{-1} (*data not shown*). All treatments averaged > 11.0 μmol CO_2 m^{-2} s^{-1} on 8 July, until falling to < 5.0 μmol CO_2 m^{-2} s^{-1}on 20 July. Gross photosynthesis was < 1.0 μmol CO_2 m^{-2} s^{-1} for all treatments on the final collection date in 2011 (8 September). Rough bluegrass treated with Heritage TL averaged greater Pg than untreated RBG on 15 June 2011, whereas Insignia SC-treated RBG averaged greater Pg than untreated RBG on 15 and 28 June 2011 (Table 3.10). In Manhattan (2012), Pg of untreated and fungicide-treated RBG averaged 10.9 to 15.8 μmol CO_2 m^{-2} s^{-1} on 4 June, and fungicide-treated RBG never averaged less than 10.2 μmol CO_2 m^{-2} s^{-1} in Manhattan (2012) (*data not shown*). Gross photosynthesis of untreated RBG was not different from any fungicide treatment until 24 July, when Pg of untreated RBG was 12.7 μmol CO_2 m^{-2} s^{-1}, significantly less than that of Heritage 50 WDG-treated RBG (17.3 μmol CO_2 m^{-2} s^{-1}) (Table 3.10). Gross photosynthesis of untreated RBG was < 6.0 μmol CO_2 m^{-2} s^{-1} by 15 August, and remained depressed through the final monitoring date (30 August). Fungicides and the respective rating dates (out of 14) on which they resulted in higher RBG Pg estimates than untreated turf were: Heritage 50 WDG (5), Heritage TL (5), Insignia 20 WG (3), and Insignia SC (4).

In Mead, respective Pg estimates (μmol CO_2 m^{-2} s^{-1}) on 28 June 2012 were: untreated (10.7), Heritage 50 WDG (18.1), Heritage TL (12.7), Insignia 20 WG (14.5), and Insignia SC (16.6) (Table 3.10). Gross photosynthesis of Heritage 50 WDG- and Insignia SC-treated RBG was significantly greater than that of untreated RBG. Among treatments, Pg was < 8.0 μmol CO_2 m^{-2} s^{-1} for all treatments on 25 July, between 9.0 and 17.0 μmol CO_2 m^{-2} s^{-1} on 28 August, and > 18.0 μmol CO_2 m^{-2} s^{-1} by the final monitoring date (26 September) with no significant differences between untreated and fungicide-treated RBG (*data not shown*). The association between maintenance of Pg and green RBG cover would suggest that fungicidal compounds did not directly affect the metabolic activity of RBG, but rather influenced Pg by delaying leaf senescence. In fact, Pg of RBG is dependent on RBG green cover according to Pearson's correlation coefficient (r=0.57, *P*=0.0001) calculated with the CORR procedure of SAS. Brosnan et al. (2010) provide excellent discussion of possible mechanisms resulting in delayed leaf senescence from applications of strobilurin fungicides. Briefly, strobilurin fungicides have been linked to ethylene inhibition and increased endogenous cytokinin, antioxidant (superoxide dismutase and peroxidase), and auxin production in wheat (Grossmann and Retzlaff, 1997; Köhle el al., 2002). Given that auxins and cytokinins regulate cell elongation and division, respectively, and that auxins and cytokinins directly stimulate shoot growth, and ethylene induces senescence and ripening (Taiz and Zeiger, 2010), it is logical that the perseverance of RBG during summer stress in this study is related to hormonal shifts encouraged by fungicide applications.

Electrolyte Leakage

Electrolyte leakage of unstressed RBG averaged 9.1 to 13.8% among treatments in Manhattan on 25 May 2011 (*data not shown*). Electrolyte leakage was never > 30.0% for any treatment in 2011. In general, there were no consistent trends in EL data. Untreated RBG had 9.2, 10.7, and 12.9% EL on 22 June, 6 July, and 20 July, respectively, significantly less than Insignia SC (13.3%), Heritage 50 WDG (13.9%), and Heritage TL (20.2%) on the same dates, respectively. Fungicides and the respective rating dates (out of 15) on which they resulted in RBG EL lower than untreated turf were: Heritage 50 WDG (3), Heritage TL (2) Insignia 20 WG (2), and Insignia SC (2). In 2012, EL was never > 32.0% for any treatment and, similar to 2011, there were no consistent trends in EL data. Untreated RBG averaged 15.9 and 10.7% EL on 26 June and 14 August, respectively, significantly less than Heritage TL (32.0%) and Heritage 50 WDG (14.3%) on the same dates, respectively. Out of 13 total rating dates, untreated RBG never had greater EL than Heritage 50 WDG-treated RBG and only averaged higher EL (19.6%) than Heritage TL- (14.2%), Insignia 20 WG- (12.2%), and Insignia SC-treated RBG (15.5%) on

25 July 2012. Similar to the growth chamber study, EL should have at least increased during periods of low RBG quality. The lack of increasing EL in this study is likely an artifact of the sampling method as previously discussed. As a result, EL was not a good indicator of RBG stress in either growth chamber or field studies, and has been unreliable in other recent studies (Rutledge et al., 2012a; Rutledge et al., 2012b).

Disease Incidence and Rooting Parameters

In Manhattan (2011), baseline sampling of RBG on 27 May 2011, prior to decline, revealed no indications of common foliar or root pathogens. Roots from all treatments appeared healthy with little, to no discoloration and all treatments averaged root health > 4.5. There were no indications of disease development between disease samplings on 27 May and 14 July, by which time RBG had severely declined. Some foliage cultured from the 14 July sampling produced mycelium that was not indicative of any common turf pathogens, and further identification was not conducted. Root health had also declined by 14 July, and untreated plots had root health of 2.8. Rough bluegrass treated with Heritage 50 WDG, Heritage TL, Insignia 20 WG, and Insignia SC had root health of 4.0, 3.8, 3.3, and 3.8, respectively, on this date, all significantly higher than untreated (Table 3.11). Small amounts of dark hyphae typical of ectotrophic root infecting fungi were present on RBG roots, resulting in lower average root health scores. The presence of ectotrophic fungi on roots, coupled with field symptomology of declining RBG, could suggest summer patch (*Magnaporthe poae* Landschoot & Jackson) or necrotic ring spot (*Ophiosphaerella korrae* [J. Walker & A. M. Sm.] Shoemaker & Babcock), but fungal signs were present at very low levels and not considered to be pathogenic. Furthermore, it is not likely that the prevention of a root-infecting pathogen led to increased root health, RBG quality, or RBG green cover because fungicides were applied to foliage, not roots. Azoxystrobin and pyraclostrobin are acropetal and localized penetrants, respectively, and cannot reach roots to protect against root-infecting pathogens without irrigation following application. Rough bluegrass is susceptible to many common turfgrass diseases, but the lack of foliar and/or root signs and symptoms suggests that none were responsible for the decline of RBG in this study.

In Manhattan (2012), samples from healthy RBG on 11 June showed no signs of typical foliar or root pathogens. All fungicide-treated turf had average roots heath scores > 4.8, and none differed from untreated RBG. There were again no indications of disease development between disease samplings, and there were no foliar signs of disease in cultures from declining RBG samples on 7 August. Root heath among treatments averaged 2.8 to 3.0, with no significant differences, and small amounts of ectotrophic fungi were present on RBG roots from all plots, regardless of treatment. Plugs removed for root analysis on 24 August revealed that RBG treated with Heritage 50 WDG had greater RLD (11.1 cm cm^{-3}), root surface area (175.2 cm^2), and root biomass (7.6 mg cm^{-2} dry weight) compared to roots from untreated RBG (RLD, surface area, and root biomass of 6.7 cm cm^{-3}, 92.1 cm^2, and 3.6 mg cm^{-2} dry weight, respectively) (Table 3.11). Root parameters from RBG treated with other fungicides were not different from untreated RBG.

Root biomass and RLD were much lower in field studies compared to growth chamber studies, which ranged between 11.7 to 14.3 mg cm^{-2} and 31.6 to 34.8 cm cm^{-3}, respectively. Rough bluegrass was established in sterile fritted clay in the growth chamber study in an attempt to isolate the effects of fungicide treatments on heat-stressed RBG. The increased porosity and/or lower bulk density of fritted clay compared to field soil, coupled with daily irrigation to field capacity in growth chambers, likely resulted in the increased rooting of RBG in the growth chamber study. The general increased rooting in growth chambers may have resulted in the lack of fungicide effects on RBG quality or root growth of heat stressed RBG compared to the Manhattan (2012) field study. The roots of most turfgrasses are not major carbohydrate storage organs (Beard, 1973), and RBG roots likely didn’t directly provide carbohydrate reserves for survival during heat stress, but rather indirectly enhanced heat tolerance by providing a continuous water supply to ensure stomata remained open for transpirational cooling. Tall fescue has superior heat tolerance among cool-season turfgrasses which largely results from its deep root system (Jiang and Huang, 2001). Root health maintenance has also been shown to directly affect turf quality, tiller density, shoot growth rate, and clipping yield of creeping bentgrass (Xu and Huang, 2001). Furthermore, cytokinins are synthesized in roots and are linked to delayed leaf senescence (Taiz and Zeiger, 2010). The greater root biomasses of RBG in the growth chamber study could have resulted in higher cytokinin content in RBG shoots delaying leaf senescence and nullifying potential fungicide effects.

Only untreated plots were sampled in Mead in 2012. Dollar spot was present on 28 June 2012 with < 10% of affected plots showing symptoms. Dollar spot was cultured from two of four untreated plots. There were no signs of root diseases on this date, and root health of RGB in untreated plots averaged 3.5. Samples from declining RBG on 27 July revealed no foliar signs or symptoms, and small amounts of ectotrophic fungi were present on untreated RBG roots. The overall dark appearance of roots resulted in an average root heath of 2.0. Analysis of RBG roots on 28 August revealed that RLD, surface area, and root biomass among treatments was 8.5 to 9.4 cm cm^{-3}, 119.4 to 129.3 cm^{2}, and 4.1 to 4.6 mg cm^{-2}, respectively, with no differences compared to untreated RBG (Table 3.11).

Results of root analysis from Manhattan (2012) and Mead are different from those of Brosnan et al. (2010) who observed increased visual root length, total root length, root surface area, RLD, root volume, and total root biomass of pyraclostrobin-treated creeping bentgrass under deficit irrigation and decreased visual root length, total root biomass, and RLD of azoxystrobin-treated creeping bentgrass under more frequent irrigation. In this study, neither increased rooting with either pyraclostrobin product, nor decreased rooting with azoxystrobin as a microemulsion concentrate (Heritage TL) was observed. Instead, I observed increased rooting from treatment with azoxystrobin (Heritage 50 WDG) compared to untreated RBG in Manhattan (2012) only.

Conclusions

The aggressive use of QoI fungicides improved RBG quality and green cover during stressful summer months, and azoxystrobin products generally resulted in healthier RBG compared to untreated RBG more consistently than did pyraclostrobin products. In general, Pg of fungicide-treated RBG did not decrease as much as untreated RBG during stress, likely resulting from delayed leaf senescence. Fungicides had little effect on RBG rooting. While treatment with Heritage 50 WDG resulted in improved rooting compared to untreated RBG in the field in Manhattan, the same was not true in Mead or the growth chamber study. Disease sampling did not reveal a fungal pathogen consistently associated with the decline of RBG. I acknowledge that RBG is susceptible to many common summer turfgrass diseases, but theorize that disease incidence is secondary, and abiotic physiological stresses are the primary reason for the summer decline of RBG. It is unclear why strobilurin fungicides positively influence RBG in the field, but I agree with Brosnan et al. (2010) that poorly understood non-target physiological effects of fungicidal compounds may likely be the foundation of this phenomenon. Still, fungicides should be used as such, and not as biostimulants. Rough bluegrass is extremely sensitive to heat, and it is still unclear if similar effects will occur on more heat-tolerant turf species without exceeding label constraints. I suggest further research with QoI fungicides and other turfgrass species within label restrictions.

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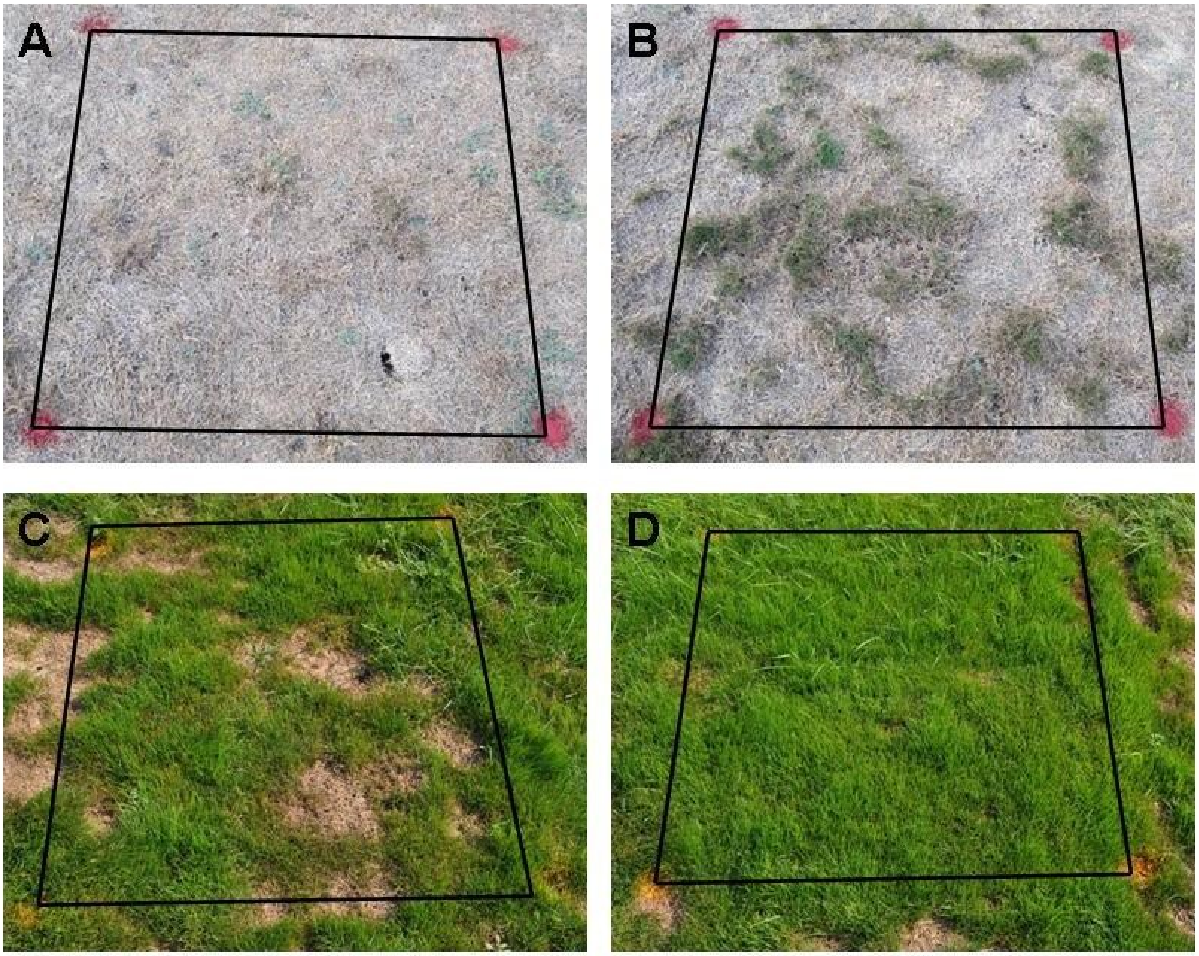

Figure 3.1 Effects of QoI fungicides on rough bluegrass quality and cover in Manhattan, KS in 2011 and 2012. A) Untreated rough bluegrass on 1 September 2011. B) Heritage TL-treated rough bluegrass on 1 September 2011. C) Untreated rough bluegrass on 28 August 2012. D) Heritage TL-treated rough bluegrass on 28 August 2012. Other fungicides yielded similar results compared to Heritage TL-treated rough bluegrass in each year.

Table 3.1 Effects of fungicide treatments on rough bluegrass quality when exposed to supraoptimal temperatures (35°C day/29°C night) in a growth chamber.

	Quality[†]					
Treatments[‡]	0 DAI[§]	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
Untreated[¶]	8.3	7.5	6.8	5.3	4.0	2.8
Heritage 50 WDG	8.3	7.8	6.8	5.5	4.3	2.5
Heritage TL	8.5	7.5	6.8	6.0	4.5	2.8
Insignia 20 WG	8.8	7.3	7.0	6.3	4.5	3.0
Insignia SC	8.3	7.3	6.5	6.0	4.3	3.0

[†] Turfgrass quality was rated visually considering color, density, and uniformity on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity).

[‡] Fungicide treatments were applied 7 days prior to heat treatment (-7 days of treatment), and at 7 and 21 days after induction of heat treatment (DAI) with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa. The experiment was set up in a randomized complete block design with four replications, with one pot per fungicide treatment in each replication.

[§] Days after induction (DAI) of heat treatment.

[¶] A set of single-degree-of-freedom contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.2 Effects of fungicide treatments on gross photosynthesis (Pg) of rough bluegrass exposed to supraoptimal temperatures (35°C day/29°C night) in a growth chamber.

	Pg (μmol CO_2 m^{-2} s^{-1})[†]					
Treatments[‡]	0 DAI[§]	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
Untreated[¶]	10.9	7.3	5.1	3.2	3.4	2.6
Heritage 50 WDG	10.4	7.5	5.0	4.0	3.0	2.5
Heritage TL	10.9	7.5	5.4	3.4	2.8	2.7
Insignia 20 WG	10.2	7.6	5.9	4.8 *	3.4	2.7
Insignia SC	11.9	8.4	5.4	4.3	3.5	2.9

[†] Gross photosynthesis was estimated from the sum of sunlit and shaded measurements taken with a portable photosynthesis chamber.

[‡] Fungicide treatments were applied 7 days prior to heat treatment (-7 days of treatment), and at 7 and 21 days after induction of heat treatment (DAI) with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa. The experiment was set up in a randomized complete block design with four replications, with one pot per fungicide treatment in each replication.

[§] Days after induction (DAI) of heat treatment.

[¶] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.3 Effects of fungicide treatments on clipping yields of rough bluegrass exposed to supraoptimal temperatures (35°C day/29°C night) in a growth chamber.

	Clipping yield (mg cm^{-2})[†]				
Treatments[‡]	7 DAI[§]	14 DAI	21 DAI	28 DAI	35 DAI
Untreated[¶]	1.0	0.7	0.4	0.1	0.0
Heritage 50 WDG	0.9	0.8	0.4	0.1	0.0
Heritage TL	1.1	1.2	0.6	0.2	0.0
Insignia 20 WG	1.2	1.1	0.6	0.2	0.0
Insignia SC	0.9	0.7	0.4	0.1	0.0

[†] Clipping yields were estimated by collecting clippings produced weekly. Clippings were oven-dried at 60°C for two days, and then weighed.

[‡] Fungicide treatments were applied 7 days prior to heat treatment (-7 days of treatment), and at 7 and 21 days after induction of heat treatment (DAI) with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa. The experiment was set up in a randomized complete block design with four replications, with one pot per fungicide treatment in each replication.

[§] Days after induction (DAI) of heat treatment.

[¶] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.4 Effects of fungicide treatments on electrolyte leakage (EL) of rough bluegrass exposed to supraoptimal temperatures (35°C day/29°C night) in a growth chamber.

	Electrolyte leakage (%)[†]					
Treatments[‡]	0 DAI[§]	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
Untreated[¶]	24.7	13.2	15.9	18.2	26.9	24.5
Heritage 50 WDG	21.7	12.7	19.4	14.8	32.7	19.7
Heritage TL	21.4	19.0	20.5	19.2	24.7	18.9
Insignia 20 WG	20.1	12.9	14.3	16.6	27.1	24.0
Insignia SC	24.6	16.9	14.6	11.9	21.5	20.3

[†] Leaf segments were agitated for 24 hours to remove electrolytes adhering to, and released from severed plant tissue. After shaking for 24 hours, the electrical conductivity of the solution was measured, and test tubes were placed in a 90°C water bath for one hour. After agitating samples for an additional 24 hours, final electrical conductivity measurements were taken (% EL = initial electrical conductivity / final electrical conductivity × 100).

[‡] Fungicide treatments were applied 7 days prior to heat treatment (-7 days of treatment), and at 7 and 21 days after induction of heat treatment (DAI) with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa. The experiment was set up in a randomized complete block design with four replications, with one pot per fungicide treatment in each replication.

[§] Days after induction (DAI) of heat treatment.

[¶] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.5 Effects of fungicide treatments on shoot biomass, root biomass, and root length density (RLD) of rough bluegrass exposed to supraoptimal temperatures (35°C day/29°C night) in a growth chamber.

	Shoot biomass[‡]	Root biomass[§]	RLD[¶]
Treatments[†]	-------------- mg cm^{-2}	--------------	cm cm^{-3}
Untreated[#]	57.0	12.2	34.8
Heritage 50 WDG	57.0	11.7	31.6
Heritage TL	58.9	14.3	34.7
Insignia 20 WG	59.5	12.7	34.4
Insignia SC	58.9	14.3	34.2

[†] Fungicide treatments were applied 7 days prior to heat treatment (-7 days of treatment), and at 7 and 21 days after induction of heat treatment (DAI) with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa. The experiment was set up in a randomized complete block design with four replications, with one pot per fungicide treatment in each replication.

[‡] Total above-ground biomass was collected at 35 DAI, oven-dried at 60°C for two days, and weighed.

[§] At 35 DAI, a 5 cm (diameter) × 17.5 cm (depth) plug was randomly removed from each pot. Roots were washed, oven-dried at 60°C for two days, and weighed.

[¶] Root length density (RLD) was determined by analyzing clean roots from a 5 cm (diameter) × 17.5 cm (depth) plug with WinRHIZO.

[#] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.6 Effects of fungicide treatments on rough bluegrass quality in Manhattan, KS in 2011.

	Quality[†]						
Treatments[‡]	31 May	28 June	27 July	25 Aug.	28 Sept.	27 Oct.	11 Nov.
Untreated[§]	7.3	7.3	2.5	1.3	1.0	1.3	1.8
Heritage 50 WDG	7.5	7.3	3.5	2.8 **	2.0 *	2.5 *	2.8 *
Heritage TL	7.5	7.8	5.0 **	3.5 ***	2.5 **	2.3 **	3.0 *
Insignia 20 WG	7.0	7.5	3.5	2.3 *	1.5	2.0	2.3
Insignia SC	7.8	7.3	3.8	2.5 *	1.0	1.8	2.3

[†] Turfgrass quality was rated visually considering color, density, and uniformity on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity). Data were collected monthly from 31 May to 11 November in 2011.

[‡] Fungicide treatments were applied every other week from 21 May to 23 August with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa.

[§] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.7 Effects of fungicide treatments on rough bluegrass quality in Manhattan, KS and Mead, NE in 2012.

	Quality[†]											
	Manhattan							Mead				
Treatments[‡]	30 May	27 June	27 July	30 Aug.	26 Sept.	31 Oct.	13 Nov.	18 May	29 June	31 July	30 Aug.	28 Sept.
Untreated[§]	7.5	8.0	6.0	4.8	6.5	8.0	7.0	7.0	5.8	4.8	5.3	6.8
Heritage 50 WDG	7.5	8.0	6.3	7.8 ***	8.0 **	8.0	7.0	7.0	7.3 **	6.5 ***	6.8 ***	7.8 **
Heritage TL	8.3	8.0	7.5 *	8.3 ***	8.8 ***	8.5 *	7.5 **	6.8	6.8 *	6.0 ***	6.5 **	7.5 *
Insignia 20 WG	8.0 *	8.0	7.0	7.3 ***	8.5 ***	8.5 *	7.0	7.0	6.3	5.8 **	6.0 *	7.3
Insignia SC	7.3	8.0	6.8	7.5 ***	8.5 ***	8.0	7.0	7.0	6.5	6.0 ***	6.3 **	8.0 ***

[†] Turfgrass quality was rated visually considering color, density, and uniformity on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity). Data were collected on monthly from 30 May to 13 November and from 18 May to 28 September in Manhattan and Mead, respectively.

[‡] Fungicide treatments were applied every other week from 23 April to 30 August in Manhattan and from 4 May to 30 August in Mead with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa .

[§] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.8 Effects of fungicide treatments on rough bluegrass cover in Manahttan, KS in 2011.

	Green rough bluegrass cover (%)[†]							
Treatments[‡]	31 May	28 June	27 July	28 Aug.	28 Sept.	27 Oct.	11 Nov.	30 May 12
Untreated[§]	99.5	98.5	43.8	0.8	0.3	3.3	4.8	30.0
Heritage 50 WDG	99.0	98.3	48.8	16.8 **	7.5 *	16.8 *	21.3	87.8 ***
Heritage TL	99.5	99.5	77.5	21.3 **	7.8 *	14.8 *	17.5 *	91.0 ***
Insignia 20 WG	97.0	95.3	52.5	8.3	2.0	5.0	7.0	63.8
Insignia SC	99.0	97.3	63.8	7.5	1.0	6.3	7.3	63.8 *

[†] Percent green rough bluegrass cover data were collected as a visual estimate. Data were not normally distributed, and were subjected to an arcsin (y/100) transformation prior to analysis and back-transformed for presentation. Data were collected monthly from 26 May to 11 November in 2011 and again on 30 May 2012.

[‡] Fungicide treatments were applied every other week from 23 April to 30 August in Manhattan and from 4 May to 30 August in Mead with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa .

[§] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.9 Effects of fungicide treatments on rough bluegrass cover in Manahttan, KS and Mead, NE in 2012.

	Green rough bluegrass cover (%)[†]															
	Manhattan								Mead							
Treatments[‡]	30 May	27 June	27 July	30 Aug.	26 Sept.	31 Oct.	13 Nov.	24 May 13	18 May	29 June	31 July	30 Aug.	28 Sept.	24 Oct.	5 Nov.	3 June 13
Untreated[§]	95.3	95.0	77.5	73.8	78.3	95.3	93.5	99.0	78.8	67.5	58.8	62.5	71.3	73.8	75.0	92.5
Heritage 50 WDG	97.8	98.0	85.5	95.3***	95.8***	99.3**	98.3**	98.8	82.5*	81.3***	78.8***	82.5**	80.0**	80.0*	80.0	95.0*
Heritage TL	98.5	98.5	95.8**	98.0***	97.8***	100.0***	100.0***	99.5	80.0	76.3*	70.0*	83.8**	80.0**	80.0*	82.5**	95.0*
Insignia 20 WG	98.3	97.8	93.0*	93.8***	96.5***	100.0***	100.0***	98.3	78.8	78.8**	72.5**	76.3*	76.3	76.3	78.8	95.0*
Insignia SC	93.8	94.0	88.3	88.5**	92.5**	97.5	96.8*	98.3	80.0	78.8**	76.3**	80.0**	76.3	76.3	77.5	93.8

[†] Percent green rough bluegrass cover data were collected as a visual estimate. Data were not normally distributed, and were subjected to an arcsin (y/100) transformation prior to analysis and back-transformed for presentation. Data were collected monthly from 30 May to 13 November in 2012 and again on 24 May 2013 in Manhattan and from 18 May to 5 November in 2012 and again on 3 June 2013 in Mead.

[‡] Fungicide treatments were applied every other week from 23 April to 30 August in Manhattan and from 4 May to 30 August in Mead with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa .

[§] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.10 Effects of fungicide treatments on gross photosynthesis (Pg) of rough bluegrass in Manhattan, KS in 2011 and 2012 and in Mead, NE in 2012.

	Pg (µmol CO2 m^{-2} s^{-1})[†]								
	2011		2012						
	Manhattan		Manhattan						Mead
Treatments[‡]	15 June	28 June	24 July	31 July	8 Aug.	15 Aug.	21 Aug.	30 Aug.	28 June
Untreated[§]	12.8	14.4	12.7	9.1	6.2	5.9	6.7	5.9	10.7
Heritage 50 WDG	12.8	14.9	17.3 *	11.6	12.7 **	16.9 ***	17.1 **	18.8 ***	18.1 **
Heritage TL	17.4 *	16.2	15.5	14.6 *	11.3 *	14.0 **	18.5 **	14.1 **	12.7
Insignia 20 WG	15.3	13.2	16.3	15.1 *	13.1 **	10.2	13.6	17.4 ***	14.5
Insignia SC	17.0 *	17.5 *	15.8	11.1	15.3 ***	15.9 **	15.1 *	18.2 ***	16.6 *

[†] Gross photosynthesis was estimated from the sum of sunlit and shaded measurements taken with a portable photosynthesis chamber. In Manhattan (2011), Manhattan (2012), and Mead (2012) data were collected on 10 dates from 31 May to 8 September 2011, 14 dates from 4 June to 6 September 2012, and 4 dates from 28 June to 26 September, respectively. Only significant dates are shown.

[‡] Fungicide treatments were applied every other week from 23 April to 30 August in Manhattan and from 4 May to 30 August in Mead with a CO_2-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa .

[§] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

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*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3.11 Effects of fungicide treatments on rooting parameters in Manhattan, KS in 2011 and 2012 and Mead, NE in 2012.

	Manhattan					Mead			
	RLD[†]	Surface area	Root biomass	Root health[‡]		RLD	Surface area	Root biomass	Root health
Treatments[§]	cm cm^{-3}	cm^2	mg cm^{-2}	2011	2012	cm cm^{-3}	cm^2	mg cm^{-2}	2012
Untreated[¶]	6.7	92.1	3.6	2.8	2.8	8.5	119.4	4.1	2.0
Heritage 50 WDG	11.1 *	175.2 **	7.6 **	4.0 ***	3.0	9.1	127.4	4.6	-
Heritage TL	7.5	96.4	4.1	3.8 ***	2.8	9.4	127.0	4.6	-
Insignia 20 WG	8.7	119.3	4.6	3.3 *	2.8	8.9	129.3	4.6	-
Insignia SC	8.7	127.9	5.1	3.8 ***	3.0	8.5	121.0	4.6	-

[†] Root length density (RLD) and root surface area were determined by analyzing roots from a 5 cm (diameter) × 17.5 cm (depth) plug randomly removed from each plot on 24 and 28 August 2012 in Manhattan and Mead, respectively. Roots were cleaned, oven-dried at 60°C for two days, and weighed to determine root biomass.

[‡] In Manhattan, plots were sampled for the presence of pathogens when rough bluegrass had declined on 11 July 2011 and 7 August 2012. In Mead, untreated plots were sampled on 27 July 2012. Roots were rated on a 1 to 5 scale (1=completely dark/discolored; 2=mostly dark/discolored; 3=approximately 50% healthy, 50% discolored, minor incidence of ectotrophic fungi; 4=minor discoloration; and 5=healthy root system).

[§] Fungicide treatments were applied every other week from 23 April to 30 August in Manhattan and from 4 May to 30 August in Mead with a CO$_2$-powered sprayer equipped with a TeeJet XR 8008 EVS nozzle calibrated to deliver water carrier equal to 816 L ha^{-1} at 207 kPa .

[¶] A set of single-degree-of-freedom-contrasts were used to compare fungicide treatments to the untreated control.

*, **, and *** are significantly different from untreated rough bluegrass at the 0.05, 0.01, and 0.001 probability level, respectively.

Chapter 4 - Effects of Tall Fescue Seeding Rate and Mowing Height on Rough Bluegrass Encroachment During Establishment

Abstract

Rough bluegrass (RBG, *Poa trivialis* L.) is a perennial cool-season species and a weed in tall fescue (TF, *Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.) turf due to differences in summer stress tolerance between the species. Rough bluegrass is likely introduced as a seed contaminant during establishment, and chemical control strategies are limited. The objective of this study was to determine the effects of TF seeding rate and mowing height on TF/RBG establishment when RBG is included as a seed contaminant. Two separate studies were conducted at the Rocky Ford Turfgrass Research Center in Manhattan, KS. Mowing height was the whole-plot treatment factor, and seeding rate was the sub-plot treatment factor. Whole-plots were mowed at 3.8, 7.6, or 11.4 cm weekly. Sub-plots were seeded in September with TF at 195, 391, or 586 kg ha^{-1} with 1.0% RBG contamination by weight. Study 1 was evaluated in 2012 and 2013 and Study 2 was evaluated in 2013. Tall fescue establishment was acceptable with all seeding rate × mowing height combinations. Mowing TF at 7.6 or 11.4 cm reduced RBG incidence by 39 or 57%, respectively, in the second year after establishment compared to mowing at 3.8 cm. Seeding rates did not consistently influence RBG incidence, and had no effect by the end of the study. Mowing TF at > 7.6 cm can mitigate RBG encroachment, but altering TF seeding rate/mowing height will not eliminate RBG.

Introduction

Rough bluegrass (RBG, *Poa trivialis* L.) is a perennial cool-season species that often declines during summer due to sensitivity to heat and drought stresses (Beard, 1973). The species is sometimes recommended for use in shady sites, and has been used in winter overseeding programs in the southern U.S.A. Tall fescue (TF, *Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.) is commonly used in sports fields, golf course roughs, and residential lawns in the transition zone, and is the most heat and drought tolerant species among cool-season turfgrasses (Fry and Huang, 2004). Rough bluegrass is considered a weed in TF stands due to its contrasting color and texture, as well as its intolerance to biotic and abiotic stresses. Bispyribac-sodium (Velocity 17.6 SG, Valent U.S.A Corporation, Walnut Creek, CA) is the only selective postemergence herbicide currently labeled for RBG control in cool-season turfgrasses, and is effective (McCullough and Hart, 2011; Morton et al., 2009), but is only labeled for use on golf courses and sod farms (Anonymous, 2010). Glyphosate controls RBG nonselectively, and is often the only chemical control option in sports fields and residential lawns.

Naturalized populations of RBG are thought to spread vegetatively during aeration via dispersal of vegetative propagules, while improved varieties with fine texture and relatively dark green color are likely introduced directly from seed lots (Levy, 1998; Reicher et al., 2011). Weed control in seed production has become more difficult since 1990, when a mandatory change from burning to mechanical removal of post harvest residue was initiated (Mueller-Warrant, 1990; Mueller-Warrant and Rosato, 2005). In 1996, Levy (1998) tested 90 creeping bentgrass (*Agrostis stolonifera* L.) seed samples from 10 seed companies and found that 30% of seed lots contained RBG seed. Following this study, seed producers moved creeping bentgrass seed production areas away from RBG production areas and improved sanitation procedures (Reicher et al., 2011). Nonetheless, RBG contamination remains a major concern. In 2008, Reicher et al. (2011) sampled 37 cultivars/blends of creeping bentgrass from 10 distributors from five Midwestern states, the majority of which were certified. Rough bluegrass was detected in 8 of 72 seed lots. While similar seed contamination in TF has not been confirmed empirically, it is generally accepted that RBG is introduced into TF lawns and sports fields as a seed contaminant. In fact, TF seed yields have been observed to decrease with increasing RBG ground cover in production fields (Mueller-Warrant and Rosato, 2005), potentially validating contamination concerns.

Rough bluegrass is characteristically competitive during establishment, even with turfgrass species that establish quickly. When seeded intentionally in mixtures with 8 to 43% perennial ryegrass (*Lolium perenne* L.) by seed number, RBG decreased the tillering of perennial ryegrass by up to 30% (Haggar, 1979). In a separate study, RBG had a higher relative growth rate than perennial ryegrass from 25 to 81 days after emergence (Vartha, 1973). Despite rough bluegrass's aggressive growth habit, it may be possible to favor TF over RBG during establishment by altering seeding rates and/or mowing heights. For example, higher mowing heights favor TF over bermudagrass [*Cynodon dactylon* L. (Pers.)] and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.]. Mowing TF at 6 cm reduced bermudagrass encroachment over a season compared to mowing at 2 cm (Brede, 1992), whereas mowing TF at 9 cm significantly reduced the amount of smooth crabgrass establishment in a season compared to mowing at 3 cm (Dernoeden et al., 1993). Similarly, Voigt et al. (2001) observed more crabgrass (*D. spp.*) in TF mowed at 2.5 cm compared to that mowed at 5.1 or 7.6 cm. Higher initial mowing heights also favor perennial ryegrass over Kentucky bluegrass (*Poa pratensis* L.) when seeded in mixtures (Brede and Duiche, 1984). Furthermore, lawn care providers routinely seed lawns at higher than recommended rates (> 391 kg ha^{-1}), but it is not know what effect higher TF seeding rates have on RBG encroachment during establishment. Further information is needed to optimize TF seeding rate and initial mowing height to minimize RBG colonization. Therefore, the objective of this study was to determine the effects of TF seeding rate and mowing height and on TF and RBG establishment when RBG is included as a seed contaminant.

Materials and Methods

Two identical studies were conducted at the Rocky Ford Turfgrass Research Center in Manhattan, KS. Study 1 was established on a site previously covered with perennial ryegrass. The stand was treated with glyphosate [N-(phosphonomethyl)glycine; Glyphomate 41, PBI/Gordon Corporation, Kansas City, MO] on 13 August 2011. The borders and alleyways of the study area were seeded with perennial ryegrass at 391 kg ha^{-1} on 7 September 2011. Study 2 (also previously perennial ryegrass) was treated with glyphosate on 15 August 2012, and borders and alleyways were seeded on 7 September 2012. Seeding rate of TF and RBG was a treatment factor and is described below. Both studies were arranged with split-plots in randomized complete-block designs with four replications. Mowing height was the whole-plot treatment factor, and seeding rate was the sub-plot treatment factor. Whole-plots measured 1.5 × 4.5 m, and were mowed at 3.8, 7.6, or 11.4 cm weekly. Sub-plots measured 1.5 × 1.5 m and were seeded on 15 September 2011 (Study 1 – evaluated in 2012 and 2013) and 18 September 2012 (Study 2 – evaluated in 2013) with TF and 1.0% RBG by weight. Sub-plots were seeded to represent seeding rates of 195, 391, or 586 kg ha^{-1}. As such, plots were first seeded with 'Second Millennium' TF at 193, 387, or 580 kg ha^{-1}, and then seeded with 'Laser' RBG at 2, 4, or 6 kg ha^{-1}, respectively. Rough bluegrass seed was spread with 24 kg N ha^{-1} of a natural organic fertilizer (Sustane, 8-2-4 [N-P_2O_5-K_2O], Sustane Natural Fertilizer Inc., Cannon Falls, MN) to aid in spreading RBG seed evenly throughout each plot.

After seeding, N was applied to Study 1 at 49 kg ha^{-1} on 11 November 2011; 26 March, 15 May, 18 September, and 9 November 2012; and 16 April, 17 May, 19 September, and 1 November 2013. Study 2 was fertilized identically to Study 1 beginning 9 November 2012. Polymer-coated urea (41-0-0; Polyon/Pursell Industries, Sylacauga, AL) was used on 15 May 2012 and 17 May 2013, and urea (46-0-0) was used on all other dates. Dimension 2 EW [dithiopyr: S,S'-dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioate; Dow AgroSciences LLC, Indianapolis, IN] and Speed Zone {Carfentrazone-ethyl: Ethyl α,2-dichloro-5-[4(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate; 2,4-D, 2-ethylhexyl ester: 2,4-dichlorophenoxy acetic acid equivalent; Mecoprop-p acid: (+)-R-2-(2-methyl-4-chlorophenoxy)propionic acid equivalent; and Dicamba acid: 3,6-dichloro-o-anisic acid equivalent; PBI/Gordon Corporation, Kansas City, MO} were applied at 0.6 and 1.2 kg a.i. ha^{-1}, respectively, on 26 March 2012 and 9 May 2013 for common dandelion (*Taraxacum officinale* Wigg.) control and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Muhl.] and large crabgrass [*D. sanguinalis* (L.) Scop.] prevention. Additionally, Merit 0.5 G {Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine; Bayer Environmental Science, Research Triangle Park, NC} was applied at 0.4 kg a.i. ha^{-1} on 29 May 2012 and 6 June 2013 for control of southern masked chafer (*Cyclocephala lurida* Bland) and May beetle (*Phyllophaga* spp.) larvae. Both studies were irrigated to prevent drought stress.

Turfgrass quality (1 to 9 scale, 1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity) and RBG incidence were rated for Study 1 on 31 May, 31 July, 30 September, and 27 November 2012, and for studies 1 and 2 on 31 May, 30 July, 20 September, and 13 November 2013. Rough bluegrass incidence was determined by rating RBG frequency with presence/absence counts under an 81-intersection grid that measured 0.9 × 0.9 m with 10 cm between each of nine gridlines in either direction (% RBG incidence = RBG frequency/81 × 100) (Figure 4.1). Brown patch (*Rhizoctonia solani* Kuhn) was observed in 2012 and 2013 in both studies and was rated as percent of plot blighted by brown patch symptoms when present.

Data Analysis

Residual normality was tested with the *w* statistic of the Shapiro-Wilk test using the UNIVARIATE procedure of Statistical Analysis System (SAS Institute Inc., Cary, NC) (Shapiro and Wilk, 1965). Brown patch severity data were not normally distributed and were subject to an arcsin (*y*) transformation prior to analysis. All data were subject to analysis of variance using the GLIMMIX procedure of SAS. Fisher's protected LSD ($P \leq 0.05$) was used to detect treatment differences.

Results and Discussion

In each study, all mowing height × seeding rate treatment combinations resulted in > 98% ground cover by the spring following seeding, and there were no significant differences among treatment combinations or main effects according to visual estimates (*data not shown*). There was never a mowing height × seeding rate interaction for any parameter evaluated, and main effects of mowing height and seeding rate in each study will be discussed separately for RBG incidence, turfgrass quality, and brown patch severity.

Rough Bluegrass Incidence

Study 1

Mowing height affected RBG incidence, but not until the second year after seeding (2013). Mowing at 3.8, 7.6, or 11.4 cm resulted in 64, 68, or 71% RBG incidence, respectively, eight months after seeding in May of 2012, with no significant differences among mowing heights (Table 4.1). Rough bluegrass incidence remained relatively unchanged throughout 2012 and into June of 2013, when mowing at 3.8, 7.6, or 11.4 cm resulted in 74, 77, or 61% RBG incidence, respectively, again, with no significant differences among mowing heights. However, by September of 2013, RBG incidence had declined to < 30% in plots mowed at 11.4 cm, significantly less than plots mowed at 3.8 cm (54% RBG incidence). Higher mowing continued to reduce RBG incidence and by the final rating date in November of 2013, mowing at 7.6 or 11.4 cm reduced RBG incidence compared to mowing at 3.8 cm. It is well documented that mowing TF higher mitigates bermudagrass and crabgrass encroachment (Brede, 1992; Dernoeden et al., 1993; Voigt et al., 2001), but studies specifically investigating the effects of mowing height on RBG encroachment in TF stands during establishment are limited. Perennial ryegrass, another bunchgrass, is also more competitive with sod-forming grasses with higher initial mowing heights. Brede and Duich (1984) observed that perennial ryegrass/Kentucky bluegrass seed mixtures mowed at 3.8 cm required no less than 95% Kentucky bluegrass seed to produce a 50:50 stand two months after seeding, while mixtures mowed at 1.3 cm required only 50 to 75% Kentucky bluegrass for a 50:50 stand. In this study, RBG incidence among mowing heights was similar until the fall of the second year after seeding (2013).

Seeding rate affected RBG incidence during the first year after seeding (2012), but had no effect after May in the second year after seeding (2013). Seeding at 195 kg ha^{-1} in September 2011 resulted in greater RBG incidence (73%) in May 2012 than seeding at 586 kg ha^{-1} (63%) (Table 4.1). In September 2012, November 2012, and May 2013, plots that had been seeded at 195 kg ha^{-1} had less RBG than those seeded at 586 kg ha^{-1}. Rough bluegrass incidence then began to decline regardless of seeding rate, and there were no differences among seeding rates in July, September, or November of 2013.

Study 2

Similar to Study 1, mowing height generally had no effect the first year after seeding (2013) in Study 2. Rough bluegrass incidence ranged from 51 to 57% among mowing heights in May of 2013 and from 69 to 75% in July of 2013, with no differences among mowing heights on either rating date (Table 4.2). By September, mowing at 11.4 cm reduced RBG incidence to 33%, significantly less than turf mowed at 3.8 or 7.6 cm. However, mowing height again had no effect in November, when RBG incidence ranged from 45 to 57% among mowing heights.

Seeding rates had no effect the first year after seeding (2013) in Study 2. In May, seeding at 391 or 586 kg ha^{-1} resulted in 60 and 56% RBG incidence, respectively, significantly more than seeding at 195 kg ha^{-1} (45 % RBG) (Table 4.2). There were no other differences among seeding rates in Study 2 in 2013, and all seeding rates averaged 52% RBG incidence on the final rating date in November of 2013.

Turfgrass Quality

Study 1

Mowing height had no effect on turf quality the first (2012) or second (2013) years after seeding Study 1. Mowing at 3.8 cm resulted in lower turfgrass quality compared to higher mowing heights in May 2012 and 2013, but quality was acceptable (> 6.0) regardless of mowing height (Table 4.3). Due to increased brown patch in higher mowing heights (Table 4.5), turf quality in plots mowed at 7.6 or 11.4 cm had unacceptable quality in July 2012, whereas turf mowed at 3.8 cm had acceptable quality. Turfgrass quality was acceptable regardless of mowing height in September and November of 2012, and July, September, and November of 2013, with no significant differences among mowing heights.

Seeding at 586 kg ha^{-1} resulted in higher turf quality than seeding at 195 kg ha^{-1} in May of 2012 (Table 4.3). There were no other differences in turf quality among seeding rates in 2012 or 2013.

Study 2

Mowing height did not consistently affect turfgrass quality the first year after seeding (2013) in Study 2 and all mowing heights resulted in acceptable quality in 2013. Turf mowed at 7.6 or 11.4 cm had higher quality than that mowed at 3.8 cm in May and September 2013 (Table 4.4).

Seeding at 195, 391, or 586 kg ha^{-1} resulted in quality of 7.6, 7.1, and 6.6, respectively, in July and all seeding rates were different from one another (Table 4.4). Turf quality was never less than acceptable in 2013, and there were no other differences in turf quality among seeding rates.

Brown Patch Severity

Brown patch was observed in Study 1 in 2012 and 2013, and in Study 2 in 2013, but statistical differences were only observed in Study 1 the first year after seeding (2012) (Table 4.5). On 5 July 2012, turf mowed at 7.6 cm had significantly more brown patch (7%) compared to turf mowed at 3.8 cm (4%). By 18 July, mowing at 7.6 or 11.4 cm resulted in more brown patch (> 18%) compared to turf mowed at 3.8 cm (4%). There were no differences in brown patch development among seeding rates. The literature is conflicting concerning brown patch development in response to mowing heights. Burpee (1995) reported increased brown patch of TF mowed at 8.9 cm compared to 3.8 cm during maximum disease development during one season, but the trend reversed the following season. Fidanza and Dernoeden (1996) observed more brown patch of perennial ryegrass mowed at 1.7 cm compared to 4.5 cm in the first year of their study, but brown patch was more severe at 4.5 cm in the second and third years of the study. In this experiment, brown patch was more severe at higher mowing heights during only the first year after seeding Study 1 (2012). The inconsistent response of brown patch to mowing heights suggests other factors are more important than canopy height for disease development.

Conclusions

Tall fescue establishment was acceptable with all seeding rates, mowing heights, and their combination. Even though RBG incidence was relatively high at the beginning of each study, it was difficult to observe without close inspection until characteristic patches began to develop in the second year after seeding. Mowing TF at 7.6 or 11.4 cm reduced RBG incidence compared to mowing at 3.8 cm, especially in the second year after establishment. Seeding rates did not consistently influence RBG incidence, and had no effect by the end of the study. Brown patch was more severe at higher mowing heights during peak disease development, but seeding rate did not influence brown patch development. Mowing TF at > 7.6 cm may be an important strategy to mitigate RBG encroachment during establishment, even before infestations are observed. However, neither seeding rates nor mowing heights evaluated in this study eliminated RBG.

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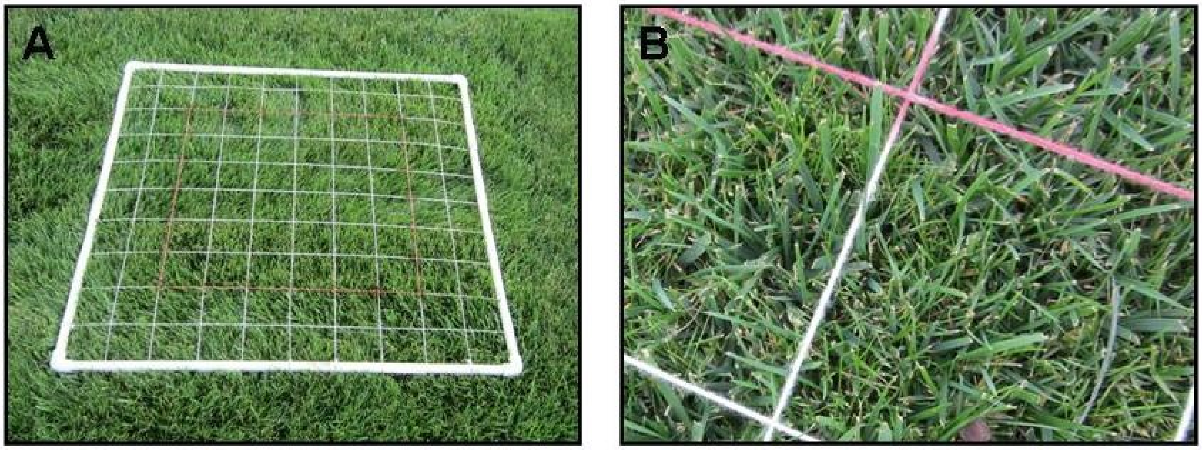

Figure 4.1 A) Transect grid used to determine rough bluegrass frequency and B) tall fescue and rough bluegrass present under intersections on 31 May 2012, approximately eight months after seeding Study 1 on 15 September 2011. The grid measured 0.9 × 0.9 m, and had 81 intersections with 10 cm between each of nine gridlines in either direction.

Table 4.1 Effect of mowing height and seeding rate on rough bluegrass incidence in Study 1 in 2012 and 2013.

	Rough bluegrass incidence (%)[†]							
	2012[‡]				2013			
Effect	May	July	Sept.	Nov.	May	July	Sept.	Nov.
Whole-plot (mowing height)[§]								
3.8 cm	64	52	68	72	71	74	54 a[¶]	61 a
7.6 cm	68	62	83	77	76	77	41 ab	37 b
11.4 cm	71	49	74	68	65	61	29 b	26 b
Sub-plot (seeding rate)[#]								
195 kg ha^{-1}	73 a	55	68 c	63 b	64 b	72	41	41
391 kg ha^{-1}	67 ab	55	75 b	76 a	69 b	69	44	41
586 kg ha^{-1}	63 b	53	82 a	78 a	77 a	71	39	40

[†] Rough bluegrass (RBG) incidence was determined by rating RBG frequency with presence/absence counts under an 81-intersection grid that measured 0.9 × 0.9 m with 10 cm between each of nine gridlines in either direction (% RBG incidence = RBG frequency/81×100).

[‡] Study 1 was evaluated in the first year after seeding (2012) on 31 May, 31 July, 30 September, and 27 November. In the second year after seeding (2013) Study 1 was evaluated on 31 May, 30 July, 20 September, and 13 November.

[§] Plots were mowed at designated heights once weekly with a rotary mower.

[¶] There was never a significant mowing height × seeding rate interaction. Means are averaged over main effects. Within columns and main effects, means are not

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significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[#] Study 1 was seeded with tall fescue on 15 September 2011 with 1.0% RBG contamination by weight.

Table 4.2 Effect of mowing height and seeding rate on rough bluegrass incidence in Study 2 in 2013.

	Rough bluegrass incidence (%)[†]			
Effect	31 May	30 July	20 Sept.	13 Nov.
Whole-plot (mowing height)[‡]				
3.8 cm	51	69	52 a[§]	57
7.6 cm	55	71	46 a	54
11.4 cm	57	75	33 b	45
Sub-plot (seeding rate)[¶]				
195 kg ha^{-1}	45 b	69	41	52
391 kg ha^{-1}	60 a	72	44	52
586 kg ha^{-1}	56 a	74	46	52

[†] Rough bluegrass (RBG) incidence was determined by rating RBG frequency with presence/absence counts under an 81-intersection grid that measured 0.9 × 0.9 m with 10 cm between each of nine gridlines in either direction (% RBG incidence = RBG frequency/81×100).

[‡] Plots were mowed at designated heights once weekly with a rotary mower.

[§] There was never a significant mowing height × seeding rate interaction. Means are averaged over main effects. Within columns and main effects, means are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[¶] Study 2 was seeded with tall fescue on 18 September 2012 with 1.0% RBG contamination by weight.

Table 4.3 Effect of mowing height and seeding rate on turf quality in Study 1 in 2012 and 2013.

	Turf quality[†]							
	2012[‡]				2013			
Effect	May	July	Sept.	Nov.	May	July	Sept.	Nov.
Whole-plot (mowing height)[§]								
3.8 cm	7.6 b[¶]	6.8 a	7.5	7.0	6.4 b	6.4	7.5	8.6
7.6 cm	8.8 a	5.9 b	7.7	7.0	8.0 a	7.1	8.2	9.0
11.4 cm	9.0 a	5.6 b	7.9	6.8	8.3 a	7.2	8.1	9.0
Sub-plot (seeding rate)[#]								
195 kg ha^{-1}	8.3 b	6.3	7.8	7.0	7.6	7.0	7.9	8.9
391 kg ha^{-1}	8.4 ab	5.9	7.7	6.8	7.7	6.8	7.8	8.9
586 kg ha^{-1}	8.7 a	6.2	7.6	7.0	7.5	6.8	8.1	8.8

[†] Turfgrass quality was rated on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity).

[‡] Study 1 was evaluated in the first year after seeding (2012) on 31 May, 31 July, 30 September, and 27 November. In the second year after seeding (2013) Study 1 was evaluated on 31 May, 30 July, 20 September, and 13 November.

[§] Plots were mowed at designated heights once weekly with a rotary mower.

[¶] There was never a significant mowing height × seeding rate interaction. Means are averaged over main effects. Within columns and main effects, means are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[#] Study 1 was seeded with tall fescue on 15 September 2011 with 1.0% RBG

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contamination by weight.

Table 4.4 Effect of mowing height and seeding rate on turf quality in Study 2 in 2013.

	Turf quality[†]			
Effect	31 May	30 July	20 Sept.	13 Nov.
Whole-plot (mowing height)[‡]				
3.8 cm	7.0 b[§]	6.6	7.3 b	8.0
7.6 cm	8.5 a	7.4	8.3 a	8.3
11.4 cm	8.2 a	7.3	8.1 a	7.6
Sub-plot (seeding rate)[¶]				
195 kg ha^{-1}	7.7	7.6 a	7.9	8.0
391 kg ha^{-1}	8.1	7.1 b	7.8	7.9
586 kg ha^{-1}	7.9	6.6 c	7.9	8.0

[†] Turfgrass quality was rated on a 1 to 9 scale (1=completely brown, 6=minimum acceptable quality, 9=optimum color, density, and uniformity).

[‡] Plots were mowed at designated heights once weekly with a rotary mower.

[§] There was never a significant mowing height × seeding rate interaction. Means are averaged over main effects. Within columns and main effects, means are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[¶] Study 2 was seeded with tall fescue on 18 September 2012 with 1.0% RBG contamination by weight.

Table 4.5 Effect of mowing height and seeding rate on brown patch severity in Study 1 in 2012 and 2013 and Study 2 in 2013.

	Brown patch severity (%)[†]				
	Study 1				Study 2
	2012			2013	2013
Effect	5 July	18 July	30 Aug.	26 July	26 July
Whole-plot (mowing height)[‡]					
3.8 cm	4 b[§]	4 b	0	6	7
7.6 cm	7 a	18 a	1	11	8
11.4 cm	5 ab	23 a	1	11	4
Sub-plot (seeding rate)[¶]					
195 kg ha^{-1}	5	11	0	8	3
391 kg ha^{-1}	6	16	0	8	6
586 kg ha^{-1}	6	17	1	12	9

[†] Brown patch severity was visually rated as percent of plot blighted by brown patch symptoms when present. Data were not normally distributed and were subject to an arcsin (*y*) transformation to normalize prior to analysis.

[‡] Plots were mowed at designated heights once weekly with a rotary mower.

[§] There was never a significant mowing height × seeding rate interaction. Means are averaged over main effects. Within columns and main effects, means are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[¶] Studies 1 and 2 were seeded with tall fescue on 15 September 2011 and 18

September 2012, respectively, with 1.0% RBG contamination by weight.

Chapter 5 - Evaluation of Selective Herbicides and Paclobutrazol on Rough Bluegrass

Abstract

Rough bluegrass (RBG, *Poa trivialis* L.) is a problematic weed in cool-season turfgrasses, and more selective postemergence control options are needed. Our objective was to evaluate amicarbazone, mesotrione, and paclobutrazol, applied alone and in combination, for efficacy against RBG and safety on desirable cool-season turfgrasses in greenhouse and field settings. In the greenhouse study, sod pieces of RBG, creeping bentgrass (*Agrostis stolonifera* L.), Kentucky bluegrass (*Poa pratensis* L.), and perennial ryegrass (*Lolium perenne* L.) were removed from established field plots and planted in the greenhouse. Two applications of herbicide treatments were made to pots of each species. Field studies were conducted in 2013 in Manhattan, KS; Hutchinson, KS; and Mead, NE. Amicarbazone, mesotrione, amicarbazone + mesotrione combinations, paclobutrazol, and bispyribac-sodium were applied one to three times at approximately 10 day intervals. In the greenhouse study, no herbicide treatment resulted in unacceptable quality of Kentucky bluegrass or perennial ryegrass, and only treatments containing mesotrione resulted in unacceptable creeping bentgrass quality. Amicarbazone and mesotrione + amicarbazone reduced RBG quality, but did not reduce RBG cover. With the exception of mesotrione, all herbicide treatments reduced RBG clipping production compared to untreated RBG, but reductions were similar to those observed in the other three species. When treated with paclobutrazol or combinations including paclobutrazol, RBG had similar green color to other species. Amicarbazone reduced the lateral spread of RBG by 40%. In the field study, mesotrione and bispyribac-sodium reduced perennial ryegrass quality at 8 weeks after initial treatment. Rough bluegrass quality was consistently reduced across all three locations with two sequential applications of amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione or three sequential applications of bispyribac-sodium. Several treatments provided transient RBG control, but bispyribac-sodium was the only treatment that provided enduring RBG control (16 to 92%) across all three locations.

Introduction

Rough bluegrass (RBG, *Poa trivialis* L.) is a cool-season perennial turfgrass and a problematic weed in cool-season landscapes, golf course turfs, and athletic fields due to invasive stoloniferous growth, suboptimal color, extreme sensitivity to heat and drought, and susceptibility to diseases (Beard, 1973; Hurley, 2003). Rough bluegrass is spread vegetatively during cultivation and as weed seed due to contamination in production seed lots (Levy, 1998; Reicher et al., 2011).

Nonselective herbicides (e.g. glyphosate) can control RBG, but few postemergence herbicides are effective for selective removal in cool-season turfgrasses. Fenoxaprop effectively reduced RBG in perennial ryegrass (*Lolium perenne* L.) production fields (Mueller-Warrant, 1990) and applications of quinclorac plus 2,4-D injured RBG six weeks after treatment, whereas Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass, and tall fescue (*Festuca arundinacea* Schreb.) were not injured (Neal and Senesac, 1993). There are no other reports of RBG control with fenoxaprop or quinclorac plus 2, 4-D. The most success selectively controlling RBG has come from applications of bispyribac-sodium {2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy] benzoic acid; Velocity 17.6 SG, Valent U.S.A. Corporation, Walnut Creek, CA} or sulfosulfuron {1-[4,6-dimethoxypyrimidin-2-yl]-3-[2-ethanesulfonyl-imidazo(1,2-a)pyridine-3-yl) sulfonyl]urea; Certainty 75 WDG, Monsanto Co., St. Louis, MO}. Sulfosulfuron is no longer labeled for use in cool season turf (Anonymous, 2012a). Bispyribac-sodium is a pyrimidinylthiobenzoic acid herbicide that inhibits acetolactate synthase (ALS), blocking amino acid synthesis in sensitive plants (Lycan and Hart, 2006; Senseman, 2007). The effectiveness of bispyribac-sodium for selective postemergence RBG reduction is well documented (Askew et al., 2004; McCullough and Hart, 2011; Morton et al., 2007), but long-term studies indicate that RBG recovery ultimately limits control (McCullough and Hart, 2011). Bispyribac-sodium also safely controls RBG soon before and after seeding desirable species. Creeping bentgrass (*Agrostis stolonifera* L.), Kentucky bluegrass, and perennial ryegrass may be safely seeded as soon as two weeks after bispyribac-sodium application (Lycan and Hart, 2006), and interseeding has been shown to improve RBG control in creeping bentgrass (Rutledge et al., 2010a). After seeding, bispyribac-sodium safely controls RBG as early as 7 days after creeping bentgrass emergence (Rutledge et al., 2010b).

Even though bispyribac-sodium is effective for RBG control, its use is limited to golf course and sod farm turf (Anonymous, 2010). More control options are needed. Amicarbazone [4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5- oxo-1*H*-1,2,4-triazole-1-carboxamide; Xonerate 4 SC, Arysta LifeScience, Cary, NC] and mesotrione {2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; Tenacity 4 SC, Syngenta Crop Protection, Greensboro, NC} are selective postemergence herbicides that are labeled for use in many turfgrass sites. Amicarbazone is labeled for use on golf courses, sod farms, residential and commercial turf sites, park and recreation areas, school grounds, and other turf areas (Anonymous, 2012b). Mesotrione is labeled for use on golf courses, sod farms, athletic fields, parks, residential and commercial properties, cemeteries, airports, and lawns (Anonymous, 2011). In sensitive plants, amicarbazone inhibits photosystem II by blocking electron transport, wheraes mesotrione is a *p*-hydroxyphenylpyruvate (HPPD)-inhibiting herbicide (Elmore et al., 2013). Recently, researchers have shown that amicarbazone and mesotrione more effectively control annual bluegrass (*Poa annua* L.) when tank-mixed than when either product is applied alone (Elmore et al., 2013). Elmore et al. (2013) explain that the synergistic effects of combinations of amicarbazone and mesotrione result from coinciding increased production of toxic singlet oxygen (amicarbazone) and decreased singlet oxygen quenching due to inhibited carotenoid production (mesotrione). Paclobutrazol {(±)-(*R*,R**)-beta-[(4-chlorophenyl)methyl]-alpha- (1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; Trimmit 2 SC, Syngenta Crop Protection, Greensboro, NC} is a plant growth regulator that inhibits gibberellic acid synthesis and is commonly used for annual bluegrass suppression in creeping bentgrass fairways and putting greens (Baldwin and Brede, 2011; McCullough et al., 2005), but it is unclear if paclobutrazol suppresses RBG. Therefore, the objective of this research was to evaluate amicarbazone, mesotrione, and paclobutrazol, when applied alone and in combination, for efficacy against RBG and safety on desirable cool-season turfgrasses.

Materials and Methods

Greenhouse Study

Sod pieces (5 cm × 10 cm) (diameter × depth) of ‘Laser’ RBG, ‘Declaration’ creeping bentgrass, ‘Bedazzled’ Kentucky bluegrass, and ‘Revenge GLX’ perennial ryegrass were removed from established research plots at the Rocky Ford Turfgrass Research Center in

Manhattan, KS on 28 January 2013, planted in 12.7 × 12.7 × 30 cm (length × width × height) pots filled with field soil (Chase silt loam: 6.8 pH, 0.08 g kg^{-1} P, 0.33 g kg^{-1} K, and 2% organic matter), and placed in the Throckmorton Plant Sciences Center Greenhouse Complex at Kansas State University. The experiment was a split-plot in a randomized complete-block design with four replications. Turf species and herbicide treatment were the whole- and sub-plot treatment factors, respectively. Whole-plots in each block were comprised of eight pots of each species. As such, each pot was a sub-plot experimental unit and was either left untreated, or treated with herbicide (described below). Pots received N at 49 kg ha^{-1} from a controlled release fertilizer [Osmocote 14-14-14 (N-P_2O_5-K_2O), Everris NA Inc., Dublin, OH], were watered to field capacity every other day, and were mowed three times each week at 1.3 cm. Mean air temperature of the greenhouse and soil temperature 10 cm deep in pots was 20 (± 3.7)°C and 20 (± 1.3)°C, respectively. Plants were provided a 14 hour photoperiod. Photosynthetically active radiation (PAR) was measured with a ceptometer (LP-80 AccuPAR PAR/LAI Ceptometer, Decagon Devices, Inc., Pullman, WA) and averaged 1,185 (± 245) µmol m^{-2} s^{-1} in a horizontal plane approximately 30 cm above the turf canopy.

Herbicide treatments were applied on 1 March 2013 and 15 March 2013 with a Links spray chamber equipped with a single 8015 LP nozzle (Spraying Systems, Co., Wheaton, IL) positioned 0.8 m over the turf canopy and operating at 124 kPa to deliver 374 L spray solution ha^{-1}. Treatments included: 1) untreated; 2) paclobutrazol (0.56 kg a.i. ha^{-1}); 3) mesotrione (0.17 kg a.i. ha^{-1}); 4) amicarbazone (0.15 kg a.i. ha^{-1}); 5) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); 6) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 7) paclobutrazol (0.56 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); and 8) mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}).

Turfgrass quality (1 to 9, 1=completely brown, 6=acceptable, 9=no phytotoxicity), percent control, and clipping yield were recorded every other week. Percent control was determined by rating green turf frequency with presence/absence counts under an 81-intersection grid that measured 11.7 × 11.7 cm with 1.3 cm between each of nine gridlines in either direction (% green turf cover=green turf frequency/81 × 100). Percent control was then determined by comparing cover on each rating date to initial cover in each pot [if % cover on rating date ≥ initial % cover, then % control=0; otherwise, % control = (initial % cover – % cover on rating date) / initial % cover × 100]. Clipping yield was determined by collecting clippings produced each week. Weekly yields were oven-dried at 60°C for two days, weighed, and reported as mass per unit area (g dry weight m^{-2}) based upon the area of each pot covered by green turf. Turfgrass color (1 to 9, where 9 = dark green turf) was rated visually from six weeks after initial treatment (WAIT) to the end of the study (10 WAIT). At 10 WAIT, lateral spread (average plug diameter), leaf area index (LAI), and shoot biomass were measured. The average diameter of each plug was determined from the mean of measurements in two perpendicular directions. Leaf area index was determined directly by defoliating a 5.0 cm plug removed from the center of each pot. Brown leaves and debris were removed from samples prior to image analysis with WinRHIZO (version 2003 b, Regent Instruments, Quebec City, Canada). Remaining foliage was then harvested from pots, and all shoot biomass was oven-dried at 60°C for two days, weighed, and reported as mas per unit area (g dry weight m^{-2}). For comparison across turf species, clipping yield, shoot biomass, average diameter, and LAI data were adjusted by scaling data from each pot to the percent of the untreated pot within each block of each species on each rating date [% of untreated=(treatment parameter rating from block x on date y / untreated parameter rating from block x on date y) × 100].

Field Study

Three separate experiments were conducted in the field study. Experiments were conducted in 2013 at the Rocky Ford Turfgrass Research Center in Manhattan, KS; Prairie Dunes Country Club in Hutchinson, KS; and The John Seaton Anderson Turf Research Center in Mead, NE. Research plots (0.9 × 0.9 m in Manhattan and Hutchinson, and 1.5 × 1.5 m in Mead) were arranged in a randomized complete-block design with three replications. Studies were conducted on monostands of ‘Laser’ and ‘Winterstar’ RBG in Manhattan and Mead, respectively. The experiment in Hutchinson was conducted on a natural infestation of RBG of unknown variety and origin in mixture with an established blend of perennial ryegrass cultivars. All studies were mowed with reel mowers and irrigated as needed to prevent drought stress. In Manhattan, RBG was mowed twice each week at 1.9 cm and fertilized with N at 49 kg ha^{-1} on 16 April, 17 May, 19 September, and 1 November 2013. Polymer-coated urea (41-0-0; Polyon/ Pursell Industries, Sylacauga, AL) was used on 17 May, and urea (46-0-0) was used on all other dates. Dimension 2 EW [dithiopyr: S,S'-dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioate; Dow AgroSciences LLC, Indianapolis, IN] and

Speed Zone {Carfentrazone-ethyl: Ethyl α,2-dichloro-5-[4(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate; 2,4-D, 2-ethylhexyl ester: 2,4-dichlorophenoxy acetic acid equivalent; Mecoprop-p acid: (+)-R-2-(2-methyl-4-chlorophenoxy) propionic acid equivalent; and Dicamba acid: 3,6-dichloro-o-anisic acid equivalent; PBI/Gordon Corporation, Kansas City, MO} were applied at 0.6 and 1.2 kg a.i. ha^{-1}, respectively, on 9 May 2013 for common dandelion (*Taraxacum officinale* Wigg.) control and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Muhl.] and large crabgrass [*D. sanguinalis* (L.) Scop.] prevention. Additionally, Merit 0.5 G {Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine; Bayer Environmental Science, Research Triangle Park, NC} was applied at 0.4 kg a.i. ha^{-1} on 6 June 2013 for control of southern masked chafer (*Cyclocephala lurida* Bland) and May beetle (*Phyllophaga* spp.) larvae. The experiment in Hutchinson was mowed three times each week at 1.3 cm and fertilized with N from urea at 15 kg ha^{-1} on 13 May, 20 kg ha^{-1} on 13 September, and 49 kg ha^{-1} on 18 November 2013. In Mead, RBG was mowed three times weekly at 1.6 cm and fertilized with polymer-coated urea to provide N at 49 kg ha^{-1} on 1 May, 1 September, and 1 November 2013. Pendimethalin (N-[1-ethylpropyl]-3, 4-dimethyl-2, 6-dinitrobenzenamine) was applied in late-April 2013 at 3.4 kg a.i. ha^{-1} and Trimec Classic (Dimethylamine salt of 2,4-dichlorophenoxyacetic acid; dimethylamine salt of [+]-[R]-2-[2-methyl-4-chlorophenoxy]propionic acid; and dimethylamine salt of dicamba: 3,6-dichloro-o-anisic acid; PBI/Gordon Corporation, Kansas City, MO) was applied at 1.5 kg a.i. ha^{-1} in late-September 2013 for broadleaf weed control.

Herbicides were applied with a CO_2-powered sprayer equipped with XR TeeJet 8002 flat spray nozzles calibrated to deliver 814 L spray volume ha^{-1} at 207 kPa. Amicarbazone, mesotrione, paclobutrazol, and bispyribac-sodium were applied one to three times at approximately 10 day intervals. Rates and combinations included: 1) untreated; 2) two sequential applications of amicarbazone (0.04 kg a.i. ha^{-1}); 3) one application of amicarbazone (0.09 kg a.i. ha^{-1}); 4) two sequential applications of amicarbazone (0.09 kg a.i. ha^{-1}); 5) one application of mesotrione (0.15 kg a.i. ha^{-1}); 6) two sequential applications of mesotrione (0.15 kg a.i. ha^{-1}); 7) three sequential applications of mesotrione (0.15 kg a.i. ha^{-1}); 8) two sequential applications of amicarbazone (0.04 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 9) one application of amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 10) two sequential applications of amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 11) three sequential applications of paclobutrazol (0.28 kg a.i. ha^{-1}); and 12) three sequential applications of bispyribac-sodium (0.08 kg a.i. ha^{-1}). Specific application dates for each site are available in Table 5.1.

In Manhattan and Mead, RBG quality (1 to 9, 1=brown, 6=minimum acceptable, and 9=optimum color, density, and uniformity) was evaluated every other week from 0 to 8 weeks after initial treatment (WAIT). Percent green RBG cover was visually estimated from 0 to 16 WAIT. In Hutchinson, RBG quality and perennial ryegrass injury (0 to 100, where 0=no injury) were evaluated every other week from 0 to 8 WAIT, and percent green RBG cover was visually estimated from 0 to 12 WAIT. In each study, percent RBG control was determined in the same way as previously described in the greenhouse study [if % cover on rating date ≥ initial % cover, then % control=0; otherwise, % control = (initial % cover – % cover on rating date) / initial % cover × 100].

Data Analysis

Residual normality was tested with the w statistic of the Shapiro-Wilk test using the UNIVARIATE procedure of Statistical Analysis System (SAS Institute Inc., Cary, NC) (Shapiro and Wilk, 1965). Percent control and clipping yield data from the greenhouse study were not normally distributed and were subjected to a $\log_{10}(y+1)$ transformation prior to analysis. All data were subjected to analysis of variance using the GLIMMIX procedure of SAS. Fisher's protected LSD ($P \leq 0.05$) was used to detect treatment differences.

Results and Discussion

Greenhouse Study

With the exception of LAI, there were significant turf species × herbicide treatment interactions for all parameters evaluated (Table 5.2).

Turfgrass Quality

In general, herbicide treatments reduced the quality of RBG more than for other species and treatment with amicarbazone or mesotrione + amicarbazone most greatly reduced RBG quality (Table 5.3). No herbicide treatment resulted in unacceptable quality (<6.0) of Kentucky bluegrass or perennial ryegrass from 0 to 10 WAIT, and only treatments containing mesotrione resulted in unacceptable creeping bentgrass quality (Table 5.3). Mesotrione is labeled for creeping bentgrass control, and the adverse effects on creeping bentgrass quality in this study were expected (Anonymous, 2011). Mesotrione also reduced perennial ryegrass quality, whereas herbicide combinations including paclobutrazol most often reduced Kentucky bluegrass quality. While Kentucky bluegrass injury is not uncommon with use of plant growth regulators (Bigelow, 2012), mesotrione doesn't generally reduce perennial ryegrass quality, and only resulted in minimal phytotoxicity to perennial ryegrass when applied at or as early as four weeks before seeding at 161, 282, 343, or 565 g a.i. ha^{-1} in a greenhouse study in Washington (Williams et al., 2009). Additionally, Beam et al. (2006) observed < 20% perennial ryegrass injury after two (280 g a.i. ha^{-1}) or three (60 or 170 g a.i. ha^{-1}) sequential applications of mesotrione. With the exceptions of paclobutrazol and amicarbazone, all treatments resulted in significantly lower RBG quality ratings (5.8 to 7.3) compared to untreated RBG by 2 WAIT. Perennial ryegrass had similar quality to RBG when treated with mesotrione at 2 WAIT, and creeping bentgrass had significantly lower quality ratings compared to RBG when treated with mesotrione or combinations including mesotrione (3.5 to 4.0).

All treatments resulted in lower RBG quality compared to untreated RBG on 4 and 6 WAIT, but reduced quality from paclobutrazol or mesotrione had diminished by 8 WAIT. With the exception of creeping bentgrass treated with mesotrione, combinations including mesotrione, or paclobutrazol + amicarbazone, quality of all turfgrass species was less affected by herbicide treatments than RBG at 4 and 6 WAIT. At 8 WAIT, only creeping bentgrass treated with mesotrione, combinations including mesotrione, or paclobutrazol + amicarbazone, as well as Kentucky bluegrass treated with paclobutrazol + mesotrione or paclobutrazol + amicarbazone had similar quality to RBG receiving the same treatment. At 10 WAIT, only amicarbazone and mesotrione + amicarbazone resulted in lower RBG quality compared to untreated RBG, and quality of RBG treated with amicarbazone was still considered unacceptable (5.3). Amicarbazone did not reduce quality of other turf species at 10 WAIT, and quality from mesotrione + amicarbazone in RBG was only similar to that in creeping bentgrass. Amicarbazone injury to desirable species has been reported previously. McCullough et al. (2010) observed only subtle differences in injury of annual bluegrass and desirable turfgrass species (creeping bentgrass, perennial ryegrass, and Kentucky bluegrass) with amicarbazone applications, especially at temperatures exceeding 20°C.

Control

No treatment controlled RBG. Applications of paclobutrazol + amicarbazone provided 14% RBG control at 2 WAIT, but the treatment had no effect by 4 WAIT (Table 5.4). Paclobutrazol and mesotrione provided 8% Kentucky bluegrass control and 5% perennial ryegrass control, respectively, at 2 WAIT, with no effect thereafter. As expected, applications containing mesotrione reduced creeping bentgrass cover from 2 to 8 WAIT, but no herbicide treatment controlled creeping bentgrass by 10 WAIT. Mesotrione efficacy on creeping bentgrass is well documented (Beam et al., 2006; Branham et al., 2005; Dernoeden, et al., 2008; Jones and Christians, 2007).

Clipping Yields

From 2 to 10 WAIT, clipping yields varied among untreated creeping bentgrass (9 to 28 g m^{-2}), Kentucky bluegrass (9 to 15 g m^{-2}), perennial ryegrass (11 to 24 g m^{-2}), and RBG (10 to 24 g m^{-2}). Clipping yields of herbicide-treated RBG were not different from untreated RBG at 2 WAIT, but creeping bentgrass treated with paclobutrazol + amicarbazone and Kentucky bluegrass treated with paclobutrazol produced 56 and 48% fewer clippings than untreated turfs, respectively (Table 5.5). At 4 WAIT, RBG treated with paclobutrazol + mesotrione + amicarbazone produced 52% fewer clippings than untreated RBG, which was statistically similar to clipping reductions in creeping bentgrass, Kentucky bluegrass, and perennial ryegrass receiving the same treatment. Treatments containing paclobutrazol and/or mesotrione reduced creeping bentgrass clipping production 63 to 78% at 4 WAIT, whereas treatments containing paclobutrazol reduced Kentucky bluegrass clipping production 48 to 72% at 4 WAIT. With the exception of mesotrione, all herbicide treatments reduced RBG clipping production compared to untreated RBG by 6 WAIT. However, RBG clipping reductions from treatments containing paclobutrazol and/or mesotrione were similar or greater in magnitude (mesotrione + amicarbazone) compared to those in creeping bentgrass. Similarly, clipping reductions from treatments containing paclobutrazol were similar to those in Kentucky bluegrass, while perennial ryegrass treated with paclobutrazol or paclobutrazol + mesotrione + amicarbazone had similar reductions in clipping production compared to RBG receiving the same treatment. At 8 WAIT, only Kentucky bluegrass treated with paclobutrazol produced fewer clippings than untreated turfgrasses. Plant growth regulators such as flurprimidol or paclobutrazol have been recommended for RBG suppression in creeping bentgrass golf course fairways (Dernoeden, 2013). However, the desirable turfgrass species in this study generally experienced similar clipping reductions to RBG when treated with paclobutrazol or treatments containing paclobutrazol, indicating that paclobutrazol may be ineffective in reducing RBG populations as it has been for reducing annual bluegrass on golf course fairways and putting greens (Baldwin and Brede, 2011; McCullough et al., 2005).

Green Color

Treatments affected the level of green color of turfgrass species at 6 and 8 WAIT. On 6 and 8 WAIT, RBG treated with paclobutrazol or combinations including paclobutrazol had a significantly darker green color than untreated RBG or RBG receiving other herbicide treatments (Table 5.6). Furthermore, RBG and creeping bentgrass treated with paclobutrazol or paclobutrazol + amicarbazone had similar green color on 6 and 8 WAIT and RBG, Kentucky bluegrass, and perennial ryegrass treated with paclobutrazol, paclobutrazol + mesotrione + amicarbazone, or paclobutrazol + amicarbazone had similar green color on 6 WAIT (Figure 5.1). On 8 WAIT, treatment with paclobutrazol, paclobutrazol + mesotrione + amicarbazone, paclobutrazol + mesotrione, or paclobutrazol + amicarbazone resulted in similar green color among RBG, Kentucky bluegrass, and perennial ryegrass. Paclobutrazol is not typically used for color enhancement as is trinexapac-ethyl (Dernoeden, 2013), but routine applications may enhance RBG color in golf course fairways making the species less noticeable without close inspection and resulting in perceived RBG suppression.

Lateral Spread, Leaf Area Index, and Biomass

From the original 5.0 cm plugs, untreated creeping bentgrass, Kentucky bluegrass, perennial ryegrass, and RBG had spread to diameters of 10.0, 8.7, 6.0, and 7.5 cm, respectively, at 10 WAIT. Amicarbazone was the only treatment that reduced the lateral spread of RBG, and did so by 40% compared to untreated RBG at 10 WAIT (Table 5.7). Amicarbazone had no effect on the lateral spread of creeping bentgrass, Kentucky bluegrass, or perennial ryegrass. Treatment with mesotrione, paclobutrazol + mesotrione + amicarbazone, or mesotrione + amicarbazone reduced the lateral spread of creeping bentgrass 43 to 48% compared to untreated creeping bentgrass at 10 WAIT. The lateral spread of Kentucky bluegrass and perennial ryegrass was not affected by herbicide treatments.

At 10 WAIT, LAI of untreated creeping bentgrass, Kentucky bluegrass, perennial ryegrass, and RBG was 1.2, 1.2, 1.0, and 1.6 m^2 m^{-2}, respectively, but there was neither a significant turf species × herbicide treatment interaction, nor a significant turf species main effect for LAI at 10 WAIT (Table 5.2). Averaged over turf species, paclobutrazol and combinations including paclobutrazol increased LAI 154 to 206% compared to untreated turf (*data not shown*). Additionally, treatment with paclobutrazol or combinations including paclobutrazol resulted in significantly greater LAI compared to treatment with mesotrione or amicarbazone, while treatment with paclobutrazol alone also resulted in greater LAI compared to treatment with paclobutrazol + mesotrione.

Shoot biomass for untreated creeping bentgrass, Kentucky bluegrass, perennial ryegrass, and RBG was 118, 49, 41, and 49 g dry weight m^{-2} at 10 WAIT. No herbicide treatment reduced RBG shoot biomass compared to untreated RBG, and treatment with paclobutrazol + mesotrione increased RBG shoot biomass 104% (Table 5.7). Similarly, treatment with paclobutrazol or combinations including paclobutrazol increased perennial ryegrass shoot biomass 103 to 216% compared to untreated perennial ryegrass. Herbicide treatments had no effect on creeping bentgrass or Kentucky bluegrass shoot biomass.

With the exception of applications of mesotrione or combinations including mesotrione to creeping bentgrass, herbicide combinations evaluated in this study were generally safe on creeping bentgrass, Kentucky bluegrass, and perennial ryegrass. However, because of the lack of herbicide effects on RBG quality, control, clipping yields, and shoot biomass by 10 WAIT, herbicides and herbicide combinations evaluated were generally ineffective in controlling RBG. The exception was amicarbazone applied alone, which reduced the lateral spread of RBG, but not desirable turfgrass species.

Field Study

There were signifant herbicide × location (Manhattan, Hutchinson, or Mead) interactions in the statistical models for RBG quality and percent control, and perennial ryegrass injury was only evaluated in Hutchinson. Consequently, RBG quality, percent control, and perennial ryegrass injury data will be discussed separately for each location.

Rough Bluegrass Quality

With the exception of paclobutrazol, all herbicide treatments reduced RBG quality compared to untreated RBG in Manhattan (Appendix Table A.1). In Hutchinson, one application of mesotrione, one application of amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione, two sequential applications of amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione, or three sequential applications of bispyribac-sodium reduced RBG quality. Compared to untreated RBG in Mead, RBG quality was significantly reduced by two sequential applications of amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione or three sequential applications of bispyribac-sodium.

Perennial Ryegrass Injury

No treatment was injurious to perennial ryegrass until 4 WAIT in Hutchinson (Appendix Table A.2). At that time, two sequential applications of amicarbazone (0.04 or 0.09 kg a.i. ha^{-1}) + mesotrione or three sequential applications of bispyribac-sodium injured perennial ryegrass 8 to 15%, and were the only treatments with significantly more injury than untreated RBG (2%). By 8 WAIT, only three sequential applications of mesotrione or bispyribac-sodium injured perennial ryegrass. Mesotrione was most injurious (60%), and bispyribac-sodium was marginally less injurious (45%) to perennial ryegrass. Even though mesotrione application rates/frequency were similar to or less than those in previous experiments, the level of perennial ryegrass injury from mesotrione in this study exceeds that observed in the greenhouse study and that previously reported by other researchers (Beam et al., 2006; Williams et al., 2009). Because post application temperature and irradiance levels have been shown not to affect mesotrione injury of perennial ryegrass (McCurdy et al., 2008), potential differences in injury caused by mesotrione in this study and that in the greenhouse study or reported by Beam et al. (2006) and Williams et al. (2009) are not likely due to confounding environmental factors.

Perennial ryegrass injury from bispyribac-sodium was also greater than reported by previous researchers. McCullough and Hart (2009) applied bispyribac-sodium twice on approximately a 21-day interval at 37, 74, 148, 222, or 296 g a.i. ha^{-1} and observed < 15% perennial ryegrass injury. Lycan and Hart (2005) applied bispyribac-sodium once at 37, 74, 111, 148, or 296 g ha^{-1} and observed < 16% perennial ryegrass injury 35 days after treatment with nearly complete recovery by 70 days after treatment. The efficacy of bispyribac-sodium often increases with warmer temperatures (~24 to 30°C) (Askew et al., 2004; Morton et al. 2007).

Daily maximum temperatures in Hutchinson exceeded 24°C from 0 to 12 WAIT (*data not shown*), possibly contributing to perennial ryegrass injury.

The level of mesotrione- and bispyribac-sodium-related perennial ryegrass injury in this study could also be an artifact of the rating method. On later rating dates, perennial ryegrass injury was rated as percent plot inury (0 to 100) excluding intital RBG coverage. Annual bluegrass was also present in some research plots at the beginning of the study, but percent annual bluegrass coverage was not recorded. Mesotrione and bispyribac-sodium have efficacy against annual bluegrass (Askew et al., 2004; Elmore et al., 2013). Therefore, the percent of plot injury excluding initial RBG coverage could include annual bluegrass injury, artificially inflating estimates of perennial ryegrass injury.

Rough Bluegrass Control

No treatment completely eliminated RBG. Several treatments provided transient RBG control from 2 to 8 WAIT in Manhattan, but only two sequential applications of amicarbazone at 0.04 or 0.09 kg a.i. ha^{-1}, two or three sequential applications of mesotrione, or three sequential applications of bispyribac-sodium enhanced RBG control compared to untreated RBG by the final rating 16 WAIT (Table 5.8). Bispyribac-sodium provided 92% RBG control at this time, and only two sequential applications of amicarbazone at 0.09 kg a.i. ha^{-1} provided similar control (63%). While RBG control from other amicarbazone treatments, mesotrione treatments, and amicarbazone + mesotrione combinations were not statistically different from that provided by two sequential applications of amicarbazone (0.09 kg a.i. ha^{-1}), control from these treatments was not different from the untreated RBG. Furthermore, RBG control from a single treatment with amicarbazone/mesotrione was not enhanced by combining the two products as has been reported with annual bluegrass (Elmore et al., 2013).

In Hutchinson, several treatments enhanced RBG control from 2 to 8 WAIT, but only three sequential bispyribac-sodium applications provided significant RBG control (58%) compared to untreated RBG by the final rating 12 WAIT. Less overall control was observed in Mead. While two sequential applications of mesotrione or three sequential applications of bispyribac-sodium controlled RBG 14 and 20%, respectively, by 12 WAIT, only bispyribac-sodium controlled RBG compared to untreated RBG by the final rating 16 WAIT. Bispyribac-sodium was not as effective in this study as it was for Morton et al. (2007), who observed up to 100% RBG control 12 WAIT in Indiana with four sequential applications at 0.07 kg a.i. ha^{-1}.

The efficacy of bispyribac-sodium in this study was similar to that reported by McCullough and Hart (2011), who observed transient RBG control for up to three months after initial treatment with three applications at 0.07 kg a.i. ha^{-1} before RBG cover was similar to the untreated.

Conclusions

Amicarbazone + mesotrione combinations did not effectively control RBG. Bispyribac-sodium was the only herbicide treatment that consistently reduced RBG coverage. The safety of bispyribac-sodium was not evaluated on creeping bentgrass or Kentucky bluegrass, but bispyribac-sodium was injurious to perennial ryegrass in this study. Bispyribac-sodium is typically less injurious to perennial ryegrass than observed in this study, but practitioners should expect at least minor reductions in perennial ryegrass quality with repeated bispyribac-sodium applications. Routine paclobutrazol applications could result in darker green RBG that is less noticeable in desirable species without close inspection.

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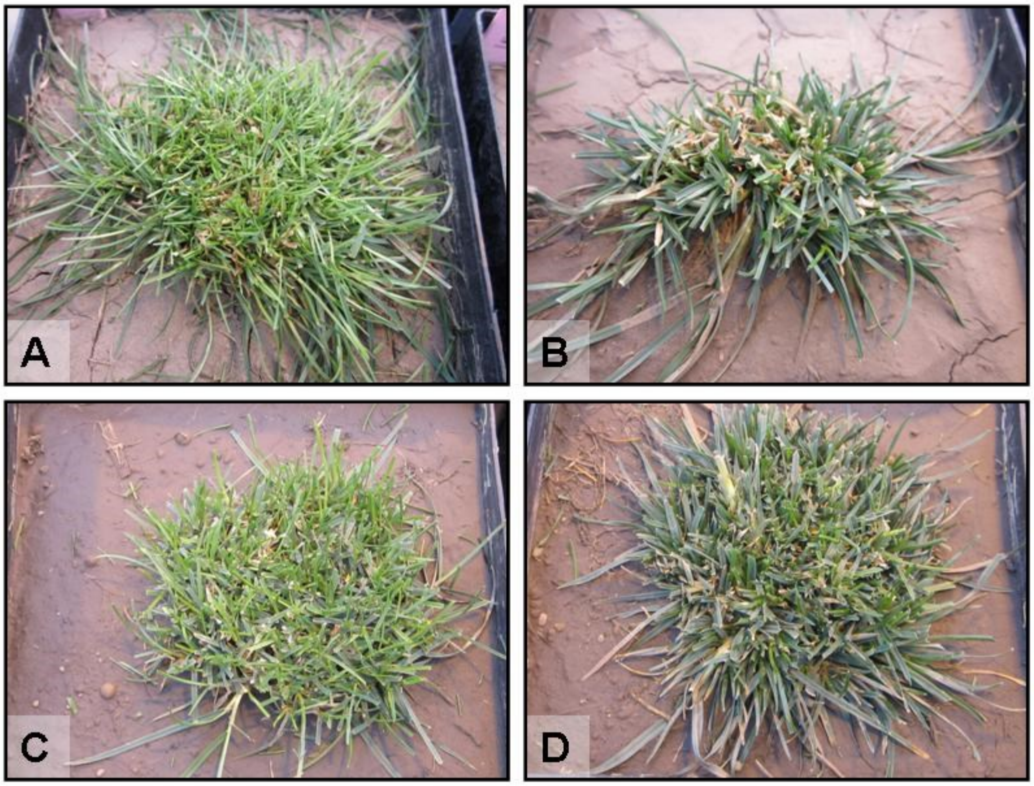

Figure 5.1 Effects of paclobutrazol on rough bluegrass (photos A and B) and perennial ryegrass (photos C and D) color six weeks after initial treatment in the greenhouse study. Rough bluegrass and perennial ryegrass in photos B and D received two applications of paclobutrazol (0.56 kg a.i. ha^{-1}), while turf in photos A and C was left untreated.

Table 5.1 Date of herbicide applications in Manhattan, KS; Hutchinson, KS; and Mead, NE in 2013.

Treatment	Rate (kg a.i. ha^{-1})[†]	Location		
		Manhattan, KS	Hutchinson, KS	Mead, NE
1) Untreated	n/a	n/a	n/a	n/a
2) Amicarbazone	0.04	A, B[‡]	A, B[§]	A, B[¶]
3) Amicarbazone	0.09	A	A	A
4) Amicarbazone	0.09	A, B	A, B	A, B
5) Mesotrione	0.15	A	A	A
6) Mesotrione	0.15	A, B	A, B	A, B
7) Mesotrione	0.15	A, B, C	A, B, C	A, B, C
8) Amicarbazone +	0.04	A, B	A, B	A, B
mesotrione	0.15			
9) Amicarbazone +	0.09	A	A	A
mesotrione	0.15			
10) Amicarbazone +	0.09	A, B	A, B	A, B
mesotrione	0.15			
11) Paclobutrazol	0.28	A, B, C	A, B, C	A, B, C
12) Bispyribac-sodium	0.08	A, B, C	A, B, C	A, B, C

[†] Herbicide treatments were applied with a CO_2-powered sprayer equipped with XR TeeJet 8002 flat spray nozzles calibrated to deliver 814 L spray volume ha^{-1} at 207 kPa.

[‡] In Manhattan, application dates were A) 27 June, B) 8 July, and C) 18 July 2013.

[§] In Hutchinson, application dates were A) 2 July, B) 15 July, and C) 29 July 2013.

[¶] In Mead, application dates were A) 27 June, B) 10 July, and C) 22 July 2013.

Table 5.2 Analysis of variance for parameters evaluated after turfgrass species were treated with herbicides in a greenhouse study in 2013.

		Weeks after initial treatment (WAIT)					
Parameter	Source of variation	0	2	4	6	8	10
Phytotoxicity	T[†]	NS[¶]	***	***	***	***	**
	H[‡]	NS	***	***	***	***	**
	T × H[§]	NS	***	***	***	***	***
Percent control[#]	T	NS	***	***	***	*	NS
	H	NS	***	***	***	NS	NS
	T × H	NS	***	***	***	**	NS
Turf color	T	n/a[††]	n/a	n/a	***	***	**
	H	n/a	n/a	n/a	***	***	***
	T × H	n/a	n/a	n/a	***	**	NS
Clipping yield[#]	T	n/a	NS	NS	NS	NS	NS
	H	n/a	*	***	***	***	***
	T × H	n/a	*	*	***	*	NS
Biomass	T	n/a	n/a	n/a	n/a	n/a	*
	H	n/a	n/a	n/a	n/a	n/a	***
	T × H	n/a	n/a	n/a	n/a	n/a	**
Plug diameter	T	n/a	n/a	n/a	n/a	n/a	*
	H	n/a	n/a	n/a	n/a	n/a	NS
	T × H	n/a	n/a	n/a	n/a	n/a	***
Leaf area index	T	n/a	n/a	n/a	n/a	n/a	NS

	H	n/a	n/a	n/a	n/a	n/a	***
	T × H	n/a	n/a	n/a	n/a	n/a	NS

[†] Turfgrass species (T).

[‡] Herbicide treatment (T).

[§] Interaction of turfgrass species and herbicide treatment combination (T × H).

[¶] Not significant (NS).

[#] Log$_{10}$(y+1) transformed prior to analysis.

[††] Not applicable (n/a).

*, **, and *** are significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 5.3 Effects of herbicide treatments on quality of turfgrass species in a greenhouse study in 2013.

		Quality[†]					
Turf species[‡]	Herbicide[§]	0 WAIT[¶]	2 WAIT	4 WAIT	6 WAIT	8 WAIT	10 WAIT
Creeping bentgrass	Untreated	9.0	9.0 a[#]	9.0 a	9.0 a	9.0 a	9.0 a
	Paclobutrazol (PB)	9.0	9.0 a	8.8 ab	8.8 ab	8.3 abc	8.0 abc
	Mesotrione (ME)	9.0	3.5 g	2.0 h	1.8 h	2.5 h	3.5 f
	Amicarbazone (AM)	9.0	8.5 ab	9.0 a	8.8 ab	8.5 ab	8.8 a
	PB + ME + AM	9.0	4.0 g	2.3 h	2.3 h	4.3 g	6.5 b-e
	PB + ME	9.0	4.0 g	2.8 h	2.8 h	4.8 fg	5.8 de
	PB + AM	9.0	8.3 ab	8.5 abc	7.0 c-g	7.3 b-e	7.5 a-d
	ME + AM	9.0	3.8 g	2.3 h	2.5 h	4.5 g	5.8 de
Kentucky bluegrass	Untreated	9.0	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a
	Paclobutrazol	9.0	9.0 a	8.5 abc	8.3 abc	8.3 abc	8.3 abc
	Mesotrione	9.0	8.8 ab	8.8 ab	9.0 a	9.0 a	9.0 a
	Amicarbazone	9.0	8.8 ab	9.0 a	8.0 a-d	9.0 a	8.5 ab
	PB + ME + AM	9.0	9.0 a	8.5 abc	7.0 c-g	7.0 b-e	7.8 a-d
	PB + ME	9.0	8.8 ab	8.3 a-d	8.0 a-d	7.3 b-e	8.0 abc

	PB + AM	9.0	8.5 ab	8.3 a-d	7.5 b-f	7.3 b-e	7.3 a-e
	ME + AM	9.0	8.8 ab	8.5 abc	7.8 a-e	8.0 abc	7.5 a-d
Perennial ryegrass	Untreated	9.0	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a
	Paclobutrazol	9.0	8.8 ab	9.0 a	9.0 a	9.0 a	9.0 a
	Mesotrione	9.0	6.3 ef	6.5 g	8.5 ab	9.0 a	9.0 a
	Amicarbazone	9.0	9.0 a	8.8 ab	8.5 ab	8.5 ab	8.5 ab
	PB + ME + AM	9.0	7.0 de	8.0 b-e	8.0 a-d	8.5 ab	8.8 a
	PB + ME	9.0	8.0 bc	8.0 b-e	8.0 a-d	8.0 abc	8.5 ab
	PB + AM	9.0	8.3 ab	8.3 a-d	7.5 b-f	8.0 abc	8.3 abc
	ME + AM	9.0	7.0 de	7.5 def	8.3 abc	8.5 ab	8.5 ab
Rough bluegrass	Untreated	9.0	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a
	Paclobutrazol	9.0	8.0 bc	7.5 def	7.0 c-g	7.8 a-d	8.5 ab
	Mesotrione	9.0	6.0 f	8.0 b-e	7.0 c-g	8.5 ab	9.0 a
	Amicarbazone	9.0	8.8 ab	7.8 c-f	5.8 g	5.8 efg	5.3 ef
	PB + ME + AM	9.0	6.0 f	7.5 def	6.3 fg	6.8 cde	7.3 a-e
	PB + ME	9.0	6.3 ef	7.5 def	6.8 d-g	7.0 b-e	7.3 a-e

PB + AM	9.0	7.3 cd	7.3 efg	6.5 d-g	7.3 b-e	8.0 abc
ME + AM	9.0	5.8 f	7.0 fg	6.0 g	6.3 def	6.3 cde

[†] Turfgrass quality was visually estimated on a 1 to 9 scale (1=completely brown, 6=acceptable, 9=no phytotoxicity) every other week from 0 to 10 weeks after initial treatment (WAIT).

[‡] Turf species was the whole-plot treatment factor. Sod pieces of ‘Laser’ rough bluegrass, ‘Declaration’ creeping bentgrass, ‘Bedazzled’ Kentucky bluegrass, and ‘Revenge GLX’ perennial ryegrass were removed from established research plots on 28 January 2013 and planted in 12.7 × 12.7 × 30 cm (length × width × height) pots filled with field soil.

[§] Herbicide treatment was the sub-plot treatment factor and products were applied on 1 March 2013 (0 WAIT) and 15 March 2013. Treatments included: 1) untreated; 2) paclobutrazol (0.56 kg a.i. ha^{-1}); 3) mesotrione (0.17 kg a.i. ha^{-1}); 4) amicarbazone (0.15 kg a.i. ha^{-1}); 5) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); 6) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 7) paclobutrazol (0.56 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); and 8) mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}).

[¶] Weeks after initial treatment (WAIT).

[#] Within columns, means with the same letter are not significantly different according to Fisher’s Protected LSD ($P \leq 0.05$).

Table 5.4 Effects of herbicide treatments on control of turfgrass species in a greenhouse study in 2013.

		Control (%)[†]					
Turf species[‡]	Herbicide[§]	0 WAIT[¶]	2 WAIT	4 WAIT	6 WAIT	8 WAIT	10 WAIT
Creeping bentgrass	Untreated	0	0 d[#]	0 c	0 c	0 c	0
	Paclobutrazol (PB)	0	0 d	0 c	0 c	0 c	0
	Mesotrione (ME)	0	57 a	77 a	64 a	34 a	18
	Amicarbazone (AM)	0	0 d	0 c	0 c	0 c	0
	PB + ME + AM	0	54 a	77 a	59 a	24 b	24
	PB + ME	0	57 a	63 b	38 b	3 bc	2
	PB + AM	0	0 d	0 c	0 c	0 c	0
	ME + AM	0	56 a	72 a	42 a	10 bc	4
Kentucky bluegrass	Untreated	0	0 d	0 c	0 c	0 c	0
	Paclobutrazol	0	8 c	0 c	0 c	0 c	0
	Mesotrione	0	0 d	0 c	0 c	0 c	0
	Amicarbazone	0	0 d	0 c	0 c	0 c	0
	PB + ME + AM	0	0 d	0 c	5 c	0 c	0

	PB + ME	0	1 cd	0 c	0 c	0 c	0
	PB + AM	0	2 cd	0 c	2 c	3 bc	12
	ME + AM	0	2 cd	0 c	0 c	0 c	0
Perennial ryegrass	Untreated	0	0 d	0 c	0 c	0 c	0
	Paclobutrazol	0	0 d	0 c	0 c	0 c	0
	Mesotrione	0	5 c	0 c	0 c	0 c	0
	Amicarbazone	0	0 d	0 c	0 c	0 c	0
	PB + ME + AM	0	0 d	0 c	0 c	0 c	0
	PB + ME	0	0 d	0 c	0 c	0 c	0
	PB + AM	0	0 d	0 c	0 c	0 c	0
	ME + AM	0	0 d	0 c	0 c	0 c	0
Rough bluegrass	Untreated	0	0 d	0 c	0 c	0 c	0
	Paclobutrazol	0	0 d	0 c	8 c	0 c	0
	Mesotrione	0	0 d	0 c	0 c	0 c	0
	Amicarbazone	0	0 d	0 c	0 c	0 c	5
	PB + ME + AM	0	0 d	0 c	0 c	0 c	0

PB + ME	0	0 d	0 c	3 c	5 bc	0
PB + AM	0	14 b	0 c	0 c	0 c	0
ME + AM	0	4 cd	0 c	5 c	0 c	0

†Percent control was determined by rating green turf frequency with presence/absence counts under an 81-intersection grid that measured 11.7 × 11.7 cm with 1.3 cm between each of nine gridlines in either direction (% green turf cover=green turf frequency/81 × 100). Percent control was then determined by comparing cover on each rating date to initial cover in each pot [if % cover on rating date ≥ initial % cover, then % control=0; otherwise, % control = (initial % cover – % cover on rating date) / initial % cover × 100]. Data were $\log_{10}(y+1)$ transformed prior to analysis and back-transformed for presentation.

‡Turf species was the whole-plot treatment factor. Sod pieces of ‘Laser’ rough bluegrass, ‘Declaration’ creeping bentgrass, ‘Bedazzled’ Kentucky bluegrass, and ‘Revenge GLX’ perennial ryegrass were removed from established research plots on 28 January 2013 and planted in 12.7 × 12.7 × 30 cm (length × width × height) pots filled with field soil.

§Herbicide treatment was the sub-plot treatment factor and were applied on 1 March 2013 (0 WAIT) and 15 March 2013. Treatments included: 1) untreated; 2) paclobutrazol (0.56 kg a.i. ha^{-1}); 3) mesotrione (0.17 kg a.i. ha^{-1}); 4) amicarbazone (0.15 kg a.i. ha^{-1}); 5) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); 6) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 7) paclobutrazol (0.56 kg a.i. ha^{-1}) + amicarbazone (0.10 kg

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a.i. ha^{-1}); and 8) mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}).

[¶] Weeks after initial treatment (WAIT).

[#] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Table 5.5 Effects of herbicide treatments on clipping yields of turfgrass species in a greenhouse study in 2013.

		Clipping yield[†]				
		---------------------------- (% of untreated)[‡]----------------------------				
Turf species[§]	Herbicide[¶]	2 WAIT[#]	4 WAIT	6 WAIT	8 WAIT	10 WAIT
Creeping bentgrass	Untreated	100 abc[††]	100 ab	100 ab	100 b-f	100
	Paclobutrazol (PB)	- 37 b-e	- 63 ghi	- 65 d-g	- 22 c-g	- 31
	Mesotrione (ME)	+ 5 ab	- 41 b-i	- 54 def	- 52 efg	+ 1
	Amicarbazone (AM)	- 26 b-e	- 22 a-g	- 20 bc	- 9 c-g	- 15
	PB + ME + AM	- 23 b-e	- 65 hi	- 87 g	- 20 c-g	- 49
	PB + ME	- 17 a-e	- 75 i	- 87 g	- 48 efg	- 33
	PB + AM	- 56 e	- 78 i	- 78 gf	- 37 c-g	- 45
	ME + AM	- 20 b-e	- 65 hi	- 80 gf	- 16 c-g	+ 3
Kentucky bluegrass	Untreated	100 abc	100 ab	100 ab	100 b-f	100
	Paclobutrazol	- 48 de	- 70 i	- 87 g	- 77 g	- 58
	Mesotrione	- 20 b-e	- 7 abc	+ 14 a	+ 86 a	+ 30
	Amicarbazone	- 15 a-e	+ 8 a	- 2 ab	+ 32 abc	+ 28

	PB + ME + AM	- 24 b-e	- 48 d-i	- 81 gf	- 47 efg	- 47
	PB + ME	- 28 b-e	- 51 d-i	- 70 efg	- 53 efg	- 36
	PB + AM	- 42 cde	- 72 i	- 74 gf	- 60 fg	- 48
	ME + AM	± 0 abc	+ 10 a	+ 7 ab	+ 28 a-d	+ 1
Perennial ryegrass	Untreated	100 abc	100 ab	100 ab	100 b-f	100
	Paclobutrazol	- 4 a-d	- 38 b-i	- 76 gf	- 35 c-g	- 17
	Mesotrione	- 14 a-e	- 21 a-f	- 21 bc	+ 38 ab	+ 34
	Amicarbazone	- 16 a-e	- 14 a-e	- 37 cd	+ 7 b-f	+ 9
	PB + ME + AM	- 32 b-e	- 57 f-i	- 73 gf	- 49 efg	- 25
	PB + ME	- 27 b-e	- 49 c-i	- 71 efg	- 39 c-g	- 28
	PB + AM	- 21 b-e	- 45 c-i	- 82 gf	- 59 fg	- 37
	ME + AM	- 10 a-d	- 24 a-h	- 18 bc	+ 13 a-e	± 0
Rough bluegrass	Untreated	100 abc	100 ab	100 ab	100 b-f	100
	Paclobutrazol	+ 8 ab	- 27 a-h	- 59 d-g	- 28 c-g	- 25
	Mesotrione	- 7 a-d	- 17 a-f	- 23 bc	+ 18 a-e	+ 30
	Amicarbazone	+ 27 a	- 10 a-d	- 39 cd	- 45 d-g	- 30

PB + ME + AM	- 10 a-d	- 52 e-i	- 68 efg	- 52 efg	- 38
PB + ME	- 21 b-e	- 40 b-i	- 58 d-g	- 41 c-g	- 1
PB + AM	+ 28 a	- 16 a-f	- 65 d-g	- 52 efg	- 31
ME + AM	+ 5 ab	- 14 a-d	- 41 cde	- 6 c-g	+ 9

[†] Weekly clipping yields were oven-dried at 60°C for two days, weighed, and reported as mass per unit area (g m^{-2} based upon the area of each pot covered by green turf.

[‡] Because clipping yields among untreated creeping bentgrass (9 to 28 g m^{-2}), Kentucky bluegrass (9 to 15 g m^{-2}), perennial ryegrass (11 to 24 g m^{-2}), and rough bluegrass (10 to 24 g m^{-2}) varied from 2 to 10 WAIT, yields were adjusted by scaling data from each pot to the percent of the untreated pot within each block of each species on each rating date [% of untreated=(treatment clipping yield from block *x* on date *y* / untreated clipping yield from block *x* on date *y*) × 100]. Data were $\log_{10}(y+1)$ transformed prior to analysis and back-transformed for presentation.

[§] Turf species was the whole-plot treatment factor. Sod pieces of ‘Laser’ rough bluegrass, ‘Declaration’ creeping bentgrass, ‘Bedazzled’ Kentucky bluegrass, and ‘Revenge GLX’ perennial ryegrass were removed from established research plots on 28 January 2013 and planted in 12.7 × 12.7 × 30 cm (length × width × height) pots filled with field soil.

[¶] Herbicide treatment was the sub-plot treatment factor and were applied on 1 March 2013 (0 WAIT) and 15 March 2013. Treatments included: 1) untreated; 2) paclobutrazol (0.56 kg a.i. ha^{-1}); 3) mesotrione (0.17 kg a.i. ha^{-1}); 4) amicarbazone (0.15 kg a.i. ha^{-1}); 5) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); 6) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 7) paclobutrazol (0.56 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); and 8) mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}).

[#] Weeks after initial treatment (WAIT).

[††] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Table 5.6 Effects of herbicide treatments on green color of turfgrass species in a greenhouse study in 2013.

		Color[†]		
Turf species[‡]	Herbicide[§]	6 WAIT[¶]	8 WAIT	10 WAIT
Creeping bentgrass	Untreated	7.0 efg[#]	7.0 efg	7.0
	Paclobutrazol (PB)	9.0 a	8.5 abc	8.5
	Mesotrione (ME)	6.0 h	5.0 i	5.0
	Amicarbazone (AM)	7.0 efg	7.0 efg	7.0
	PB + ME + AM	7.0 efg	5.7 hi	6.3
	PB + ME	6.5 gh	6.5 fgh	6.5
	PB + AM	9.0 a	8.5 abc	8.3
	ME + AM	7.0 efg	6.3 gh	6.3
Kentucky bluegrass	Untreated	7.8 cd	7.5 c-f	7.8
	Paclobutrazol	9.0 a	8.8 ab	9.0
	Mesotrione	7.3 def	7.0 efg	7.0
	Amicarbazone	7.3 def	7.0 efg	7.0
	PB + ME + AM	9.0a	8.5 abc	8.3
	PB + ME	9.0 a	8.3 a-d	7.0
	PB + AM	9.0 a	9.0 a	8.8
	ME + AM	7.0 efg	7.0 efg	7.0
Perennial ryegrass	Untreated	7.5 de	8.0 b-e	7.3
	Paclobutrazol	9.0 a	9.0 a	9.0
	Mesotrione	7.8 cd	7.5 c-f	7.8

	Amicarbazone	7.5 de	7.3 d-g	7.5
	PB + ME + AM	9.0 a	8.5 abc	9.0
	PB + ME	9.0 a	9.0 a	9.0
	PB + AM	9.0 a	8.8 ab	9.0
	ME + AM	7.5 de	7.0 efg	7.0
Rough bluegrass	Untreated	7.0 efg	7.0 efg	7.0
	Paclobutrazol	9.0 a	8.8 ab	8.5
	Mesotrione	6.8 fg	6.8 fgh	6.8
	Amicarbazone	7.0 efg	6.8 fgh	5.8
	PB + ME + AM	8.8 ab	8.3 a-d	7.8
	PB + ME	8.3 bc	8.8 ab	8.0
	PB + AM	8.5 ab	8.5 abc	8.5
	ME + AM	6.5 gh	6.8 fgh	5.8

[†] Turfgrass color (1 to 9, where 9 = dark green turf) was estimated from 6 to 10 weeks after initial treatment (WAIT).

[‡] Turf species was the whole-plot treatment factor. Sod pieces of ‘Laser’ rough bluegrass, ‘Declaration’ creeping bentgrass, ‘Bedazzled’ Kentucky bluegrass, and ‘Revenge GLX’ perennial ryegrass were removed from established research plots on 28 January 2013 and planted in 12.7 × 12.7 × 30 cm (length × width × height) pots filled with field soil.

[§] Herbicide treatment was the sub-plot treatment factor and were applied on 1 March 2013 (0 WAIT) and 15 March 2013. Treatments included: 1) untreated; 2) paclobutrazol (0.56 kg a.i. ha^{-1}); 3) mesotrione (0.17 kg a.i. ha^{-1}); 4) amicarbazone (0.15 kg a.i. ha^{-1}); 5) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i.

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ha^{-1}); 6) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 7) paclobutrazol (0.56 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); and 8) mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}).

[¶] Weeks after initial treatment (WAIT).

[#] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Table 5.7 Effects of herbicide treatments on lateral spread (average diameter), leaf area index (LAI), and shoot biomass of turfgrass species at 10 weeks after initial treatment in a greenhouse study in 2013.

		Diameter[†]	LAI[‡]	Biomass[§]
Turf species[¶]	Herbicide[#]	------------------(% of untreated)[††]---------------		
Creeping bentgrass	Untreated	100 a-f [‡‡]	100	100 e-i
	Paclobutrazol (PB)	- 5 a-f	+ 28	- 13 f-i
	Mesotrione (ME)	- 48 h	- 42	- 73 i
	Amicarbazone (AM)	- 3 a-f	+ 12	- 19 f-i
	PB + ME + AM	- 48 h	+ 3	- 73 i
	PB + ME	- 33 fgh	- 7	- 57 hi
	PB + AM	- 11 c-g	+ 44	- 26 f-i
	ME + AM	- 43 gh	+ 2	- 65 i
Kentucky bluegrass	Untreated	100 a-f	100	100 e-i
	Paclobutrazol	- 3 a-f	+ 78	+ 99 b-e
	Mesotrione	+ 2 a-e	+ 44	+ 48 b-g
	Amicarbazone	+ 1 a-f	+ 44	+ 18 d-i
	PB + ME + AM	- 25 e-h	+ 65	+ 52 b-g
	PB + ME	- 3 a-f	+ 54	+ 73 b-f
	PB + AM	- 21 d-h	+ 45	+ 54 b-g
	ME + AM	+ 1 a-e	+ 20	- 14 f-i
Perennial ryegrass	Untreated	100 a-f	100	100 e-i
	Paclobutrazol	+ 25 ab	+ 222	+ 216 a

	Mesotrione	+ 4 a-e	+ 68	+ 40 c-h
	Amicarbazone	+ 9 a-d	+ 37	+ 42 c-h
	PB + ME + AM	+ 11 a-d	+ 165	+ 125 bc
	PB + ME	+ 20 abc	+ 125	+ 142 b
	PB + AM	+ 13 abc	+ 110	+ 103 bcd
	ME + AM	+ 4 a-e	+ 26	+ 24 d-i
Rough bluegrass	Untreated	100 a-f	100	100 e-i
	Paclobutrazol	+ 11 a-d	+ 94	+ 91 b-e
	Mesotrione	+ 27 a	+ 48	+ 36 c-h
	Amicarbazone	- 40 gh	- 51	- 29 ghi
	PB + ME + AM	+ 10 a-d	+ 87	+ 37 c-h
	PB + ME	+ 2 a-e	+ 44	+ 104 bcd
	PB + AM	- 6 b-f	+ 125	+ 37 c-h
	ME + AM	- 10 c-g	- 22	- 30 ghi

[†] The average diameter of each plug was determined from the mean of measurements in two perpendicular directions and reported as a length (cm).

[‡] Leaf area index was determined directly by defoliating a 5 cm plug removed from the center of each pot. Brown leaves and debris were removed from samples prior to image analysis with WinRHIZO (version 2003 b, Regent Instruments, Quebec City, Canada). Leaf area index was reported as leaf area per unit area (m^2 m^{-2}).

[§] Shoot biomass was oven-dried at 60°C for two days, weighed, and reported as mass per unit area (g dry weight m^{-2}).

[¶] Turf species was the whole-plot treatment factor. Sod pieces of ‘Laser’ rough bluegrass,

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‘Declaration’ creeping bentgrass, ‘Bedazzled’ Kentucky bluegrass, and ‘Revenge GLX’ perennial ryegrass were removed from established research plots on 28 January 2013 and planted in 12.7 × 12.7 × 30 cm (length × width × height) pots filled with field soil.

[#] Herbicide treatment was the sub-plot treatment factor and were applied on 1 March 2013 (0 WAIT) and 15 March 2013. Treatments included: 1) untreated; 2) paclobutrazol (0.56 kg a.i. ha^{-1}); 3) mesotrione (0.17 kg a.i. ha^{-1}); 4) amicarbazone (0.15 kg a.i. ha^{-1}); 5) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); 6) paclobutrazol (0.56 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 7) paclobutrazol (0.56 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}); and 8) mesotrione (0.15 kg a.i. ha^{-1}) + amicarbazone (0.10 kg a.i. ha^{-1}).

[††] Because untreated pots of each species differed in average diameter [creeping bentgrass (10.0 cm), Kentucky bluegrass (8.7 cm), perennial ryegrass (6.0 cm), and rough bluegrass (7.5 cm)], LAI [creeping bentgrass (1.2 m^2 m^{-2}), Kentucky bluegrass (1.2 m^2 m^{-2}), perennial ryegrass (1.0 m^2 m^{-2}) and rough bluegrass (1.6 m^2 m^{-2})], and biomass [creeping bentgrass (118 g dry weight m^{-2}), Kentucky bluegrass (49 g dry weight m^{-2}), perennial ryegrass (41 g dry weight m^{-2}), and rough bluegrass (49 g dry weight m^{-2})], data were adjusted by scaling data from each pot to the percent of the untreated pot within each block of each species on each rating date [% of untreated=(treatment rating from block x on date y / untreated rating from block x on date y) × 100].

[‡‡] Within columns, means with the same letter are not significantly different according to Fisher’s Protected LSD ($P \leq 0.05$).

Table 5.8 Effect of herbicide treatments on rough bluegrass control in Manhattan, KS, Hutchinson, KS, and Mead, NE in 2013.

	Control (%)[†]													
	Manhattan, KS					Hutchinson, KS				Mead, NE				
	Weeks after initial treatment[‡]					Weeks after initial treatment				Weeks after initial treatment				
Treatment[§]	2	4	8	12	16	2	4	8	12	2	4	8	12	16
Untreated	2 f[¶]	12 f	49 e	46	17 d	0 c	0 b	29 de	0 b	3	5 b	4 bc	3 b	3 bc
Amicarbazone (0.04) (AB)[#]	12 def	60 b-e	93 ab	71	54 bc	0 c	12 b	84 abc	0 b	7	9 b	5 bc	5 b	3 bc
Amicarbazone (0.09) (A)	13 c-f	40 ef	62 de	53	45 bcd	2 c	2 b	77 a-d	0 b	6	6 b	2 c	0 b	0 c
Amicarbazone (0.09) (AB)	24 cd	96 ab	96 ab	85	63 ab	0 c	0 b	60 a-e	0 b	2	8 b	6 bc	2 b	2 bc
Mesotrione (A)	8 def	58 cde	68 cde	65	46 bcd	0 c	0 b	10 e	0 b	2	2 b	8 bc	4 b	4 bc
Mesotrione (AB)	10 def	56 cde	76 bcd	68	56 bc	3 c	5 b	58 a-e	13 b	6	6 b	14 b	14 a	8 b
Mesotrione (ABC)	4 ef	51 de	82 a-d	76	50 bc	8 bc	0 b	89 ab	17 b	6	4 b	2 c	2 b	2 bc
AM (0.04) + ME (AB)[††]	23 cde	88 a-d	89 abc	64	42 bcd	29 a	66 a	20 e	7 b	7	5 b	1 c	1 b	1 c
AM (0.09) + ME (A)[††]	48 ab	91 abc	93 ab	73	48 bcd	18 ab	7 b	34 cde	0 b	4	2 b	2 c	0 b	0 c
AM (0.09) + ME (AB)[††]	59 a	100 a	94 ab	71	49 bcd	23 ab	66 a	42 b-e	0 b	5	7 b	2 c	3 b	3 bc
Paclobutrazol (ABC)	3 f	29 f	47 e	54	27 cd	0 c	12 b	56 a-e	23 b	2	2 b	8 bc	4 b	4 bc
Bispyribac-sodium (ABC)	32 bc	98 a	98 a	95	92 a	8 bc	60 a	100 a	58 a	2	18 a	51 a	20 a	16 a

[†] Percent green rough bluegrass (RBG) cover was visually estimated from 0 to 16 WAIT. Percent RBG control was determined by comparing cover on each rating date to initial cover in each plot [if % cover on rating date ≥ initial % cover, then % control=0; otherwise, % control = (initial % cover – % cover on rating date) / initial % cover × 100].

[‡] Each herbicide treatment was applied one to three times. Weeks after initial treatment indicates the period of time after the first application was made in Manhattan (27 June),

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Hutchinson (2 July), and Mead (27 June) in 2013.

[§] Herbicide treatments were applied with a CO_2-powered sprayer equipped with XR TeeJet 8002 flat spray nozzles calibrated to deliver 814 L spray volume ha^{-1} at 207 kPa. Amicarbazone, mesotrione, paclobutrazol, and bispyribac-sodium were applied one to three times at approximately 10 day intervals. Rates and combinations included: 1) untreated; 2) amicarbazone (0.04 kg a.i. ha^{-1}); 3) amicarbazone (0.09 kg a.i. ha^{-1}); 4) amicarbazone (0.09 kg a.i. ha^{-1}); 5) mesotrione (0.15 kg a.i. ha^{-1}); 6) mesotrione (0.15 kg a.i. ha^{-1}); 7) mesotrione (0.15 kg a.i. ha^{-1}); 8) amicarbazone (0.04 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 9) amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 10) amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 11) paclobutrazol (0.28 kg a.i. ha^{-1}); and 12) bispyribac-sodium (0.08 kg a.i. ha^{-1}).

[¶] Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[#] Applications were made on 27 June (A), 8 July (B), and 18 July (C) in Manhattan; 2 July (A), 15 July (B), and 29 July (C) in Hutchinson; and on 27 June (A), 10 July (B), and 22 July (C) in Mead in 2013.

[††] Treatment is a tank-mix of amicarbazone (AM) and mesotrione (ME).

Chapter 6 - Effect of the Seasonal Timing of Glyphosate Application on Rough Bluegrass Control

Abstract

Rough bluegrass (RBG, *Poa trivialis* L.) is a problematic weed in cultivated cool-season turfgrasses. Chemical control of RBG can be challenging as herbicides labeled for its selective removal are limited. Nonselective herbicides can eradicate RBG, and properly timed applications may offer better control. Our objective was to evaluate seasonal timing of glyphosate for nonselective control of RBG. Glyphosate was applied at 3.4 kg a.i. ha^{-1} in spring, mid-summer, or late-summer in Manhattan, KS (2011 and 2012) and Mead, NE (2012). Percent green RBG cover was visually estimated. Following glyphosate applications, green RBG coverage was reduced to 0% in all treatments in Manhattan and Mead in 2011 and 2012. Among all three experiments, spring glyphosate applications resulted in the lowest green RBG coverage (1 to 31%) the following spring, followed by late-summer applications (6 to 58%), and mid-summer applications (9 to 86%).

Introduction

Rough bluegrass (RBG, *Poa trivialis* L.) is a perennial cool-season turfgrass and a problematic weed in cool-season turf due to suboptimal color, invasive stoloniferous growth, and sensitivity to heat and drought (Beard, 1973). Rough bluegrass is thought to spread vegetatively during routine aeration and from contamination in seed lots (Levy, 1998; Reicher et al., 2011). Bispyribac-sodium {2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy] benzoic acid; Velocity 17.6 SG, Valent U.S.A. Corporation, Walnut Creek, CA} is the only product currently labeled for selective RBG removal in cool-season turf and is effective, but can damage desirable species (McCullough and Hart, 2011; Morton et al., 2007). Furthermore, bispyribac-sodium is only labeled for use on sod farms and golf courses (Anonymous, 2010).

Nonselective herbicides are often the only option for RBG control in sports fields and residential lawns, but it is unclear if efficacy varies with the seasonal timing of application as it often does with bispyribac-sodium (Askew et al., 2004; Morton et al., 2007; Rutledge et al., 2010). Rough bluegrass has been anecdotally reported to persist in sites which have been renovated with use of glyphosate [N-(phosphonomethyl)glycine] before fall seeding. Adkins and Barnes (2013) observed that spring applications of imazapic plus glyphosate were more effective controlling Kentucky bluegrass (*Poa pratensis* L.) compared to summer or fall applications, but tall fescue (*Festuca arundinacea* Schreb. Syn *Schedonorus arundinaceus* Schreb.) control was better following summer applications. It is not known how RBG responds to the seasonal timing of glyphosate application. Therefore, the objective of this study was to determine if the seasonal timing of a single glyphosate application influences RBG control.

Materials and Methods

This study was conducted at the Rocky Ford Turfgrass Research Center in Manhattan, KS and at the John Seaton Anderson Turf Research Center in Mead, NE. Research plots (0.9 × 0.9 m in Manhattan and 1.5 × 1.5 m in Mead) were arranged in a randomized complete-block design with four replications. In Manhattan, two separate studies (2011 and 2012) were conducted on ‘Laser’ RBG originally seeded in the fall of 2009. Soil was a Chase silt loam with a pH of 7.6 and phosphorous and potassium levels of 0.11 and 0.42 g kg^{-1}, respectively. In Mead, the study was conducted in 2012 only, on ‘Winterstar’ RBG originally seeded in the fall of 2010. Soil was a Tomek silty clay loam with a pH of 7.5 and phosphorous and potassium levels of 0.03 and 0.50 g kg^{-1}, respectively. Research areas were irrigated as needed to prevent drought stress and mowed at 6.3 cm once weekly with a rotary mower.

In Manhattan, N was applied at 49 kg ha^{-1} on 18 March, 2 May, 19 September, and 10 November 2011 and on 26 March, 15 May, 18 September, and 9 November 2012 to provide a total of 195 kg ha^{-1} annually. Polymer-coated urea (41-0-0 [N-P_2O_5-K_2O]; Polyon/Pursell Industries, Sylacauga, AL) was used on 2 May 2011 and 15 May 2012, and urea (46-0-0) was used on all other dates. Dimension 2 EW [dithiopyr: S,S'-dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioate; Dow AgroSciences LLC, Indianapolis, IN] and Speed Zone {Carfentrazone-ethyl: Ethyl α,2-dichloro-5-[4(difluoro methyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate; 2,4-D, 2-ethylhexyl ester: 2,4-dichlorophenoxyacetic acid equivalent; Mecoprop-p acid: (+)-R-2-(2-methyl-4-chlorophenoxy)propionic acid equivalent; and Dicamba acid: 3,6-dichloro-o-anisic acid equivalent; PBI/Gordon Corporation, Kansas City, MO} were applied at 0.6 kg a.i. ha^{-1} and 1.3 kg a.i. ha^{-1}, respectively, on 12 April 2011 and 26 March 2012 for common dandelion (*Taraxacum officinale* Wigg.) control and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Muhl.] and large crabgrass [*D. sanguinalis* (L.) Scop.] prevention. Additionally, Merit 0.5 G {Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine; Bayer Environmental Science, Research Triangle Park, NC} was applied at 0.4 kg a.i. ha^{-1} on 14 April 2011 and 29 May 2012 for control of southern masked chafer (*Cyclocephala lurida* Bland) and May beetle (*Phyllophaga spp.*) larvae.

In Mead, polymer-coated urea was used to provide N at 49 kg ha^{-1} on 1 May, 1 September, and 1 November 2012 for a total of 147 kg ha^{-1} annually. Pendimethalin (N-[1-ethylpropyl]-3, 4-dimethyl-2, 6-dinitrobenzenamine) was applied in late-April 2012 at 3.4 kg a.i. ha^{-1} and Trimec Classic (Dimethylamine salt of 2,4-dichlorophenoxyacetic acid; dimethylamine salt of [+]-[R]-2-[2-methyl-4-chlorophenoxy]propionic acid; and dimethylamine salt of dicamba: 3,6-dichloro-o-anisic acid; PBI/Gordon Corporation, Kansas City, MO) was applied at 1.5 kg a.i. ha^{-1} in late-September 2012 for broadleaf weed control.

Application Timings

Treatments consisted of three application timings: spring, mid-summer, and late-summer. Glyphosate (Glyphomate 41, PBI/Gordon Corporation, Kansas City, MO) was applied at 3.4 kg a.i. ha^{-1} with a hand-held CO_2-power sprayer equipped with XR TeeJet 8002 flat spray nozzles at 207 kPa in water carrier rate equal to 327 L ha^{-1} on each of the three timings. In Manhattan (2011), applications were made on 21 May (spring, 96% green coverage), 26 July (mid-summer, 51% green coverage), and 25 August (late-summer, 9% green coverage).

Growing degree days (GDD, base temperature = 10°C) were monitored beginning 1 January at each site each year, and were used to schedule initial spring applications in Manhattan and Mead in 2012 to match the 263 GDD that accumulated in Manhattan in 2011. In 2012, spring applications were made on 23 April in Manhattan and 4 May in Mead. Mid- and late-summer applications in 2012 were made when RBG green cover was as near as possible to that in Manhattan (2011). In 2012, mid- and late-summer applications were made in Manhattan on 27 July (mid-summer, 38% green cover) and 30 August (late-summer, 37% green cover), and on 31 July (mid-summer, 70% green cover) and 6 September (late-summer, 75% green cover) in Mead.

Data Collection and Analysis

Percent green RBG cover was visually estimated monthly. In Manhattan (2011 and 2012), and Mead (2012) data were collected weekly from 26 May 2011 to 11 November 2011, 23 April 2012 to 13 November 2012, and 4 May 2012 to 5 November 2012, respectively, and again on 30 May 2012, 24 May 2013, and 3 June 2013, respectively, to measure RBG recovery from applications. At the beginning of each study in Manhattan (2011), Manhattan (2012), and Mead (2012) treatments averaged 94 to 97%, 92 to 94%, and 78 to 80% green RBG cover, respectively, with no significant differences among treatments.

Residual normality was tested with the *w* statistic of the Shapiro-Wilk test using the UNIVARIATE procedure of Statistical Analysis System (SAS Institute Inc., Cary, NC) (Shapiro and Wilk, 1965). Percent green cover data were not normally distributed, and data were subjected to a $\log_{10}(y+1)$ transformation to normalize. Means were back-transformed for presentation. Percent green RBG cover data were subjected to analysis of variance using the GLIMMIX procedure of SAS. Fisher's protected LSD ($P \leq 0.05$) was used to detect treatment differences.

Results and Discussion

Even though green RBG cover data were collected weekly in all three studies, RBG recovery the year following treatment was of most practical interest as it indicated the level of recovery following applications the previous season. For this reason, monthly green RBG cover in untreated plots during the growing season is summarized, but comparisons among treatments were only made one-year after treatment with glyphosate.

Manhattan, KS – 2011

All treatments had nearly 100% green RBG cover in May 2011 in Manhattan, and were not different from one another. Untreated plots averaged > 90% green cover until the middle of July, but only 1% green RBG cover by 16 September. Untreated plots then began to recover, and averaged nearly 15% green cover by 15 November 2011 (Figure 6.1). On 30 May 2012, approximately one year after treatments began in 2011, untreated plots averaged nearly 80% green RBG cover. The spring glyphosate application resulted in less green RBG cover (1%) than the mid-summer application (9%), whereas RBG cover from the late-summer application was not different from that of the other timings (6%).

Manhattan, KS – 2012

All treatments had nearly 100% green RBG cover in April 2012 (Figure 6.1). Untreated plots averaged > 90% green cover well into July, until declining to approximately 23% green cover on 8 August. Rough bluegrass then began to recover and untreated plots averaged nearly 96% green cover by 8 November 2012. On 24 May 2013, approximately one year after treatments began in 2012, untreated plots averaged 100% green cover and were not different from RBG that was treated with glyphosate in mid-summer (86%). Due to relatively high plot-to-plot variability, the late-summer timing (47% green RBG cover) was also not different from untreated RBG (Figures 6.1 and 6.2). The spring glyphosate application resulted in less green RBG cover (1%) at this time than all other treatments. Adkins and Barnes (2013) observed better control of Kentucky bluegrass with spring treatments of imazapic plus glyphosate, but tall fescue control was better following summer applications. Glyphosate product labels typically state that reduced weed control may result if applied during turfgrass stress (2). Ruiter and Meinen (1998) observed decreased glyphosate absorption and translocation when black nightshade (*Solanum nigrum* L. SOLNI) was water stressed and suggested a positive correlation between plant growth rate and glyphosate efficacy. In this study, RBG was most actively growing during spring glyphosate applications, leading to improved control presumably because of increased absorption and translocation.

Mead, NE – 2012

Rough bluegrass decline was not as severe in Mead, NE in 2012 as it was in Manhattan, KS in 2011 and 2012. All treatments averaged 78 to 80% green RBG cover on 4 May 2012 and were not different from one another (Figure 6.1). Untreated plots never averaged less than 70% green cover during summer, and untreated RBG reached nearly 96% green cover by 5 November 2012. On 3 June 2013, approximately one year after treatments began in 2012, untreated plots averaged 95% green RBG cover and only the spring glyphosate application resulted in less green cover (31%), statistically. Green cover of RBG treated in mid-summer (68%) was not different from untreated cover, and green cover of RBG treated in late-summer (58%) was not different from untreated, or that treated in spring.

Conclusions

Spring-applied glyphosate consistently resulted in better RBG control than treatment in mid-summer. Glyphosate application in mid- to late-summer can temporarily reduce RBG cover, but should be applied in the spring for optimum RBG control.

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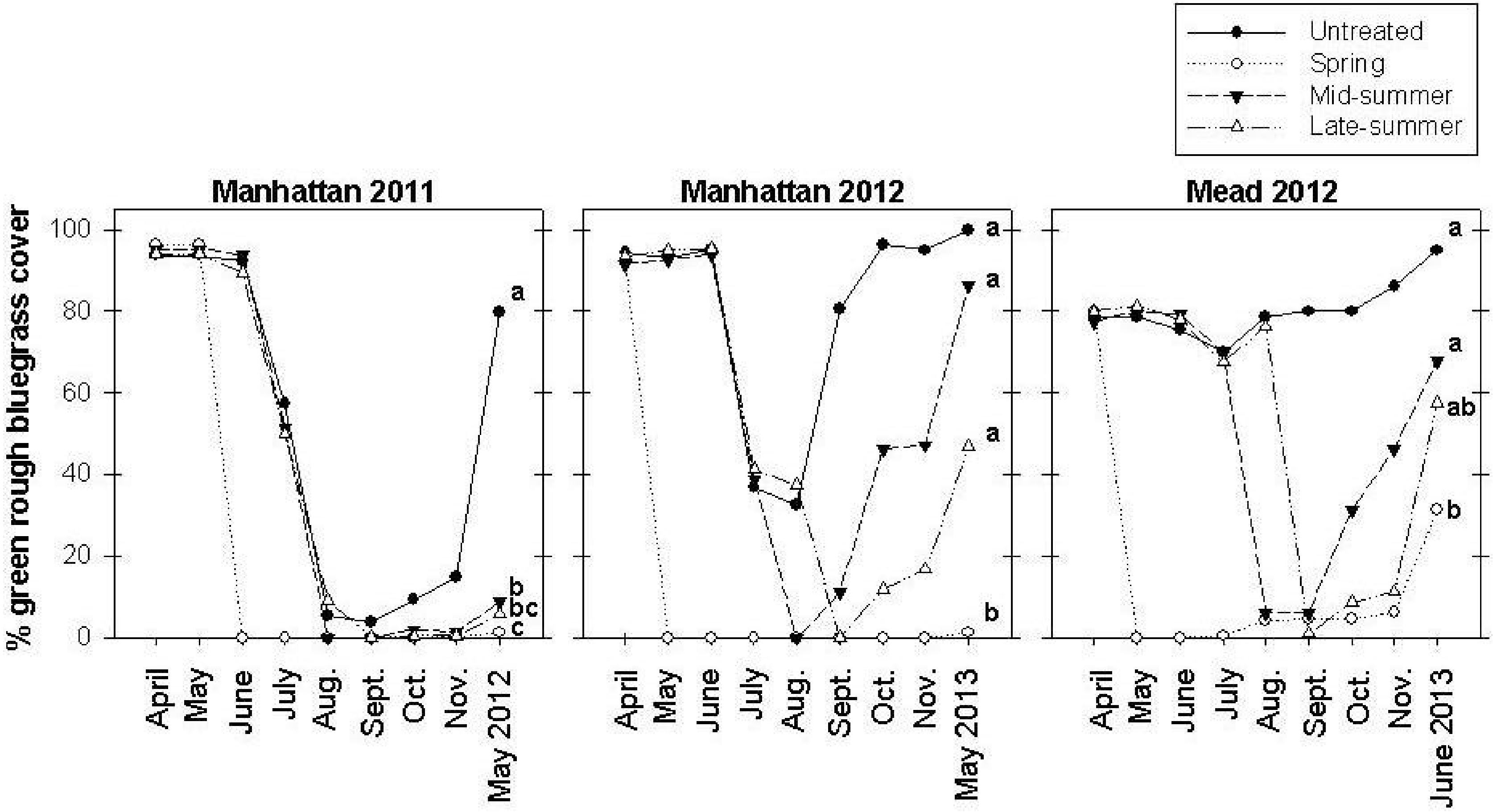

Figure 6.1 Effect of glyphosate application timing on green RBG cover in Manhattan, KS (2011 and 2012) and Mead, NE (2012). Applications were made in spring, mid-summer, or late-summer. Percent green RBG cover data were visually estimated monthly and one year after the spring applications. Data were subject to a $\log_{10}(y+1)$ transformation to normalize prior to analysis, and back-transformed for presentation. On each of the three timings, glyphosate was applied at 3.4 kg a.i.

ha^{-1} with a CO_2-powered sprayer equipped with XR TeeJet 8002 flat spray nozzles at 207 kPa in 327 L ha^{-1} spray solution. In Manhattan (2011), applications were made on 21 May (spring), 26 July (mid-summer), and 25 August (late-summer). In Manhattan (2012), application dates were 23 April (spring), 27 July (mid-summer), and 30 August (late-summer). In Mead (2012), application dates were 4 May (spring), 31 July (mid-summer), and 6 September (late-summer). There were statistical differences in RBG cover among treatments after each application and before the final rating date, but the focus here is on the final rating date, for brevity. Within each location each year, means with the same letter on the last rating date are not statistically different according to Fisher's Protected LSD (*P* ≤ 0.05).

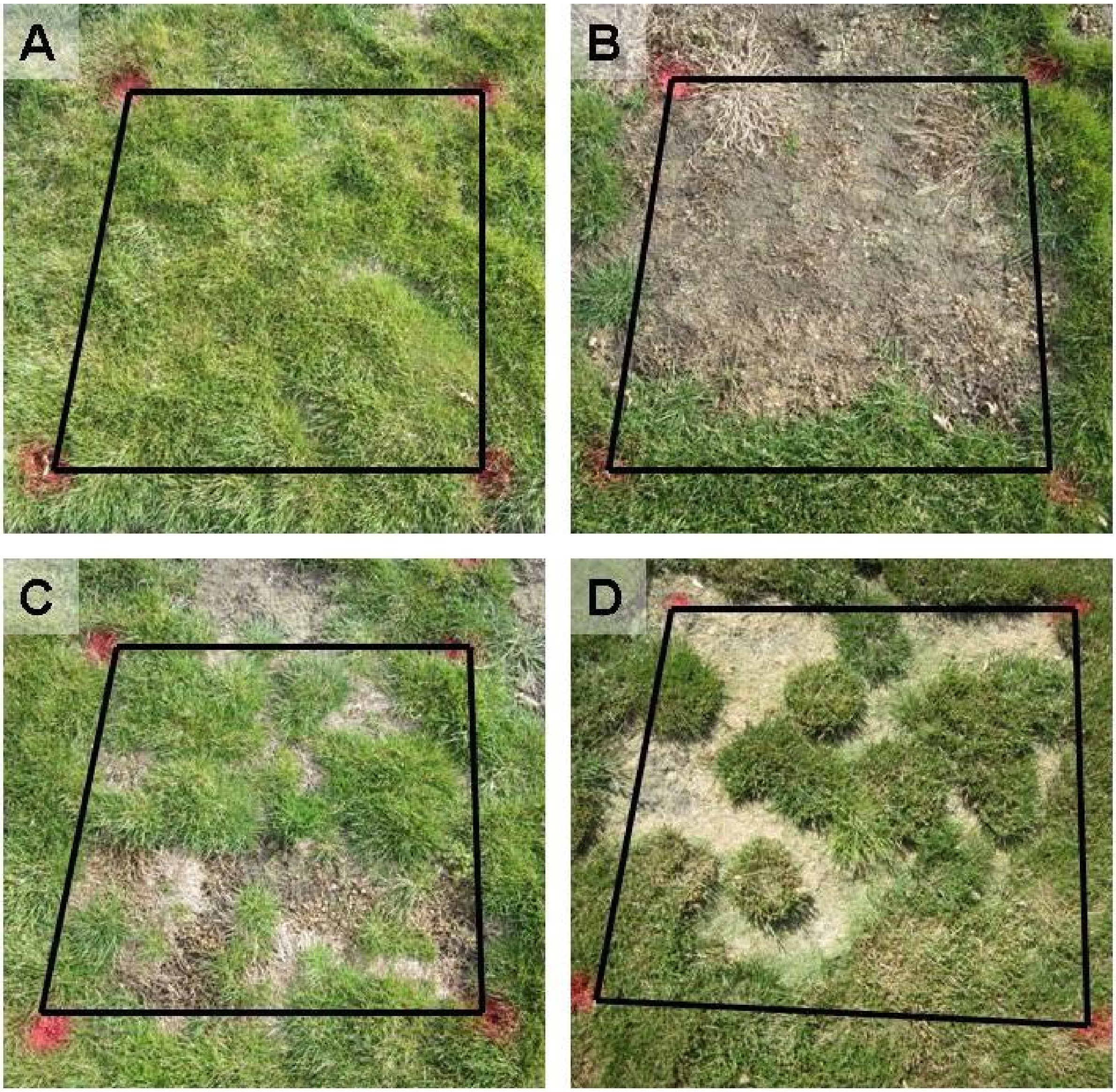

Figure 6.2 Effects of the seasonal timing of a single glyphosate application at 3.4 kg a.i. ha^{-1} on green rough bluegrass cover in Manhattan, KS on 24 May 2013. Rough bluegrass was A) untreated, or treated the year prior in B) spring (23 April 2012), C) mid-summer (27 July 2012), or D) late-summer (30 August 2012).

Appendix A - Additional Tables for Chapter 5

Table A.1 Effect of herbicide treatments on rough bluegrass quality in Manhattan, KS, Hutchinson, KS, and Mead, NE in 2013.

	Quality (%)[†]												
	Manhattan, KS					Hutchinson, KS				Mead, NE			
	Weeks after initial treatment[‡]					Weeks after initial treatment				Weeks after initial treatment			
Treatment[§]	0	1	2	4	8	0	2	4	8	0	2	4	8
Untreated	9.0	9.0 a[¶]	7.7 a	5.7 a	4.3 a	9.0	9.0 a	7.3 a	8.0	8.0	7.7	7.7 ab	6.0 abc
Amicarbazone (0.04) (AB)[#]	9.0	6.7 b	6.0 bc	2.7 cd	2.3 cde	9.0	9.0 a	7.3 a	5.7	8.0	7.0	7.0 abc	6.7 a
Amicarbazone (0.09) (A)	9.0	6.0 bc	5.7 cd	4.3 ab	3.7 ab	9.0	8.3 abc	7.3 a	5.3	8.0	6.7	6.7 bc	6.0 abc
Amicarbazone (0.09) (AB)	9.0	6.0 bc	4.3 df	1.7 de	1.3 ef	9.0	8.7 ab	7.3 a	5.0	8.0	7.3	7.3 abc	6.3 ab
Mesotrione (A)	9.0	5.7 bc	5.7 cd	4.0 bc	3.7 ab	9.0	7.0 bcd	7.0 a	7.7	8.0	7.3	8.3 a	5.3 bc
Mesotrione (AB)	9.0	6.0 bc	6.7 abc	4.0 bc	3.3 abc	9.0	8.7 ab	7.0 a	5.0	8.0	7.3	7.3 abc	5.0 c
Mesotrione (ABC)	9.0	6.0 bc	7.0 ab	4.7 ab	3.3 abc	9.0	8.0 abc	6.3 a	3.3	8.0	7.0	7.3 abc	6.7 a
AM (0.04) + ME (AB)[††]	9.0	5.7 bc	4.7 de	2.7 cd	2.7 bcd	9.0	8.0 abc	3.3 b	7.7	8.0	7.0	7.0 abc	6.3 ab
AM (0.09) + ME (A)[††]	9.0	5.7 bc	3.3 fg	2.3 de	2.3 cde	9.0	7.0 bcd	7.7 a	7.7	8.0	7.0	7.7 ab	6.3 ab
AM (0.09) + ME (AB)[††]	9.0	5.7 bc	2.7 g	1.0 e	2.0 def	9.0	6.0 d	3.3 b	7.7	8.0	6.7	6.0 c	6.0 abc
Paclobutrazol (ABC)	9.0	8.7 a	7.7 a	5.0 ab	4.3 a	9.0	8.0 abc	7.3 a	5.7	8.0	7.7	7.3 abc	5.7 abc
Bispyribac-sodium (ABC)	9.0	4.7 c	3.7 efg	1.0 e	1.0 f	9.0	6.7 cd	2.7 b	1.0	8.0	6.7	4.3 d	3.3 d

[†] Rough bluegrass quality (1 to 9, 1=brown, 6=minimum acceptable, and 9=optimum color, density, and uniformity) was evaluated every other week from 0 to 8 weeks after initial treatment.

[‡]Each herbicide treatment was applied one to three applications. Weeks after initial treatment indicates the period of time after the first application was made in Manhattan (27 June), Hutchinson (2 July), and Mead (27 June) in 2013.

[§]Herbicide treatments were applied with a CO_2-powered sprayer equipped with XR TeeJet 8002 flat spray nozzles calibrated to deliver 814 L spray volume ha^{-1} at 207 kPa. Amicarbazone, mesotrione, paclobutrazol, and bispyribac-sodium were applied one to three times at approximately 10 day intervals. Rates and combinations included: 1) untreated; 2) amicarbazone (0.04 kg a.i. ha^{-1}); 3) amicarbazone (0.09 kg a.i. ha^{-1}); 4) amicarbazone (0.09 kg a.i. ha^{-1}); 5) mesotrione (0.15 kg a.i. ha^{-1}); 6) mesotrione (0.15 kg a.i. ha^{-1}); 7) mesotrione (0.15 kg a.i. ha^{-1}); 8) amicarbazone (0.04 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 9) amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 10) amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 11) paclobutrazol (0.28 kg a.i. ha^{-1}); and 12) bispyribac-sodium (0.08 kg a.i. ha^{-1}).

[¶]Within columns, means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

[#]Applications were made on 27 June (A), 8 July (B), and 18 July (C) in Manhattan; 2 July (A), 15 July (B), and 29 July (C) in Hutchinson; and on 27 June (A), 10 July (B), and 22 July (C) in Mead in 2013.

[††]Treatment is a tank-mix of amicarbazone (AM) and mesotrione (ME).

Table A.2 Effect of herbicide treatments on perennial ryegrass injury in Hutchinson, KS in 2013.

	Injury[†]			
Treatment[‡]	0 WAIT[§]	2 WAIT	4 WAIT	8 WAIT
Untreated	0.0	0.0	1.7 d[¶]	0.0 c
Amicarbazone (0.04) (AB)[#]	0.0	0.0	1.7 d	0.0 c
Amicarbazone (0.09) (A)	0.0	0.0	3.3 cd	0.0 c
Amicarbazone (0.09) (AB)	0.0	0.0	1.7 d	1.7 c
Mesotrione (A)	0.0	0.0	3.3 cd	0.0 c
Mesotrione (AB)	0.0	0.0	7.7 bcd	0.0 c
Mesotrione (ABC)	0.0	0.0	6.7 cd	60.0 a
AM (0.04) + ME (AB)[††]	0.0	0.0	13.3 ab	0.0 c
AM (0.09) + ME (A)[††]	0.0	0.0	3.3 cd	0.0 c
AM (0.09) + ME (AB)[††]	0.0	0.0	15.0 a	0.0 c
Paclobutrazol (ABC)	0.0	0.0	3.3 cd	11.7 c
Bispyribac-sodium (ABC)	0.0	0.0	8.3 bc	45.0 b

[†] Perennial ryegrass injury (0 to 100, where 0=no injury) was evaluated every other week from 0 to 8 weeks after initial treatment.

[‡] Herbicide treatments were applied with a CO_2-powered sprayer equipped with XR TeeJet 8002 flat spray nozzles calibrated to deliver 814 L spray volume ha^{-1} at 207 kPa. Amicarbazone, mesotrione, paclobutrazol, and bispyribac-sodium were applied one to three times at approximately 10 day intervals. Rates and combinations included: 1) untreated; 2) amicarbazone (0.04 kg a.i. ha^{-1}); 3) amicarbazone (0.09 kg

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a.i. ha^{-1}); 4) amicarbazone (0.09 kg a.i. ha^{-1}); 5) mesotrione (0.15 kg a.i. ha^{-1}); 6) mesotrione (0.15 kg a.i. ha^{-1}); 7) mesotrione (0.15 kg a.i. ha^{-1}); 8) amicarbazone (0.04 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 9) amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 10) amicarbazone (0.09 kg a.i. ha^{-1}) + mesotrione (0.15 kg a.i. ha^{-1}); 11) paclobutrazol (0.28 kg a.i. ha^{-1}); and 12) bispyribac-sodium (0.08 kg a.i. ha^{-1}).

[§] Each herbicide treatment was applied one to three applications. Weeks after initial treatment (WAIT) indicates the period of time after the first application was made in Manhattan (27 June), Hutchinson (2 July), and Mead (27 June) in 2013.

[¶] Within columns, means with the same letter are not significantly different according to Fisher’s Protected LSD ($P \leq 0.05$).

[#] Applications were made on 27 June (A), 8 July (B), and 18 July (C) in Manhattan; 2 July (A), 15 July (B), and 29 July (C) in Hutchinson; and on 27 June (A), 10 July (B), and 22 July (C) in Mead in 2013.

[††] Treatment is a tank-mix of amicarbazone (AM) and mesotrione (ME).