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Herbicide Safeners Increase Creeping Bentgrass (*Agrostis stolonifera*) Tolerance to Pinoxaden and Affect Weed Control

Matthew T. Elmore, James T. Brosnan, Gregory R. Armel, Jose J. Vargas, and Gregory K. Breeden*

The herbicide pinoxaden is a phenylpyrazoline inhibitor of acetyl coenzyme A carboxylase. Research was conducted to determine the effects of pinoxaden (90 g ai ha^{-1}) alone and in combination with herbicide safeners on creeping bentgrass injury as well as perennial ryegrass and roughstalk bluegrass control. Greenhouse experiments determined that herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were more effective in reducing creeping bentgrass injury from pinoxaden than benoxacor, isoxadifen-ethyl, and naphthalic-anhydride. Other experiments determined that creeping bentgrass injury from pinoxaden decreased as rates (0, 23, 45, 68, 90, 225, or 450 g ha^{-1}) of cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl increased. On the basis of creeping bentgrass responses to various safener rates, safeners were applied at 68 and 450 g ha^{-1} in additional experiments to evaluate their effects on pinoxaden (90 g ha^{-1}) injury to creeping bentgrass and efficacy against perennial ryegrass and roughstalk bluegrass. In these experiments, safeners mefenpyr-diethyl and cloquintocet-mexyl reduced pinoxaden-induced creeping bentgrass injury (from 25 to $\leq$ 5%) more than fenchlorazole-ethyl at 2 wk after treatment. Safeners reduced pinoxaden efficacy against roughstalk bluegrass. Perennial ryegrass was controlled $>$ 80% by pinoxaden and herbicide safeners did not reduce control. Field experiments should evaluate pinoxaden in combination with cloquintocet-mexyl and mefenpyr-diethyl to optimize safener : herbicide ratios and rates for creeping bentgrass safety as well as perennial ryegrass and roughtstalk bluegrass control in different climates and seasons.

Nomenclature: Cloquintocet-mexyl; fenchlorazole-ethyl; mefenpyr-diethyl; pinoxaden; creeping bentgrass, *Agrostis stolonifera* L. AGSST; perennial ryegrass, *Lolium perenne* L. LOLPE; roughstalk bluegrass, *Poa trivialis* L. POATR

Key words: Acetyl coenzyme A carboxylase; ACCase; graminicide; phenylpyrazoline; safener; turfgrass.

El herbicida pinoxaden es una inhibidor phenylpyrazoline de la encima acetyl coenzyme A carboxylase. Se realizó una investigación para determinar los efectos de pinoxaden (90 g ai ha^{-1}) solo o en combinación con antídotos de herbicidas sobre el daño en el césped *Agrostis stolonifera* además del control de *Lolium perenne* y *Poa trivialis.* Los experimentos de invernadero determinaron que los antídotos cloquintocet-mexyl, fenchlorazole-ethyl, y mefenpyr-diethyl fueron más efectivos en reducir el daño en *A. stolonifera* causado por pinoxaden que benoxacor, isoxadifen-ethyl, y naphthalic-anhydride. Otros experimentos determinaron que el daño en *A. stolonifera* causado por pinoxaden disminuyó al aumentar las dosis (0, 23, 45, 68, 90, 225, ó 450 g ha^{-1}) de cloquintocet-mexyl, fenchlorazole-ethyl, y mefenpyr-diethyl. Con base en las respuesta de *A. stolonifera* a varias dosis de antídotos, se aplicaron antídotos a 68 y 450 g ha^{-1} en experimentos adicionales para evaluar sus efectos en el daño por pinoxaden (90 g ha^{-1}) en *A. stolonifera* y la eficacia contra *L. perenne* y *P. trivialis.* En estos experimentos los antídotos mefenpyr-diethyl y cloquintocet-mexyl redujeron el daño inducido por pinoxaden en *A. stolonifera* (de 25 a $\leq$ 5%) más que fenchlorazole-ethyl a 2 semanas después del tratamiento. Los antídotos redujeron la eficacia de pinoxaden para el control de *P. trivialis.* Sin embargo, *L. perenne* fue controlado >80% con pinoxaden y los antídotos no redujeron el control. Experimentos de campo deberían evaluar pinoxaden en combinación con cloquintocet-mexyl y mefenpyr-diethyl para optimizar la proporción de antídoto:herbicida y las dosis para la seguridad en *A. stolonifera* además del control de *L. perenne* y *P. trivialis* en diferentes climas y temporadas.

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

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

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

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

Aryloxyphenoxypropionate ACCase-inhibiting herbicides have increased selectivity in cereals when applied with the safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl (Hatzios and Burgos 2004). These safeners are known to increase conjugation of the herbicidally active ACCase inhibitor fenoxaprop acid with glutathione in wheat and barley, but not weeds such as wild oats and crabgrass (Yaacoby et al. 1991). However, not all weeds are unaffected by safeners; fenchlorazole-ethyl and mefenpyr-diethyl can reduce fenoxaprop-ethyl efficacy against blackgrass (*Alopecurus myosuroides* Huds.) (Cummins et al. 2009). In turfgrass, cloquintocet-mexyl can increase CBG tolerance to the *p*-hydroxyphenylpyruvate dioxygenase herbicide topramezone (Elmore et al. 2015). However, no other reports of increased turfgrass tolerance to herbicides from compounds used exclusively as herbicide safeners exist.

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

Table 1. Safeners applied in experiment 1 to 'Penncross' creeping bentgrass in a glasshouse in Knoxville, TN in 2013. Safeners were applied at 450 g ha^{-1}, 30 min before pinoxaden treatment at 90 g ha^{-1} to all plants except for the nontreated control.

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

[a] Safeners were applied with nonionic surfactant at 0.25% v/v.

[b] Abbreviations: N/A, not applicable; WDG, water-dispersible granule; EC, emulsifiable concentrate.

Materials and Methods

Plant Culture. Glasshouse experiments were conducted from 2012 to 2014 in Knoxville, TN (35.95°N) under ambient light. All plants were irrigated as needed to prevent wilt and grown in a peat moss, perlite, and vermiculite growing medium (Fafard no. 2, Sun Gro Horticulture, Agawam, MA) and allowed to mature for at least 6 mo before treatment.

Experiment 1: Identify Candidate Safeners. 'Penncross' CBG was seeded to 6.5-cm-diam cone-tainers (Stuewe and Sons, Tangent, OR) and maintained in a greenhouse at a 1.3-cm height using mechanical shears (Showmaster, Oster Professional Products, McMinnville, TN) for 6 mo before treatment. During this time, CBG was fertilized on an as-needed basis to maintain density and vigor using a complete (20–20–20 N–P–K) fertilizer (Howard Johnson's Triple Twenty Plus Minors, Milwaukee, WI) at 25 kg N ha^{-1} and irrigated as needed to prevent wilt. CBG had completely covered the surface of the 6.5-cm-diam cone-tainer surface and was highly stoloniferous for several months before treatment. Beginning 10 d before treatment application, CBG was fertilized with the complete fertilizer listed above at 10 kg N ha^{-1} every 10 d for the duration of the experiment. Plants were hand trimmed to a 1.3-cm height with scissors approximately 24 h before treatment and clipping was then suspended for the duration of the experiment.

CBG was treated with safeners listed in Table 1 at a 5 : 1 safener : herbicide ratio 30 min before application of pinoxaden (90 g ha^{-1}; 0.83 emulsifiable concentrate [EC], Syngenta Professional Products, Greensboro, NC). Treatments were arranged in a completely randomized single-factor treatment design with three replications. Comparisons were made with a nontreated control. Pinoxaden and the safener were applied separately to efficiently use a limited supply of pinoxaden. Safeners in Table 1 were also applied to CBG alone at 750 g ha^{-1} to assess phytotoxicity. All safener and herbicide treatments were applied with nonionic surfactant (Activator 90. Loveland Products Inc., Loveland, CO) at 0.25% v/v in 215 L ha^{-1} of water carrier through a single flat-fan nozzle (8004 EVS; Spraying Systems Co., Roswell, GA) in a spray chamber (Generation III Research Track Sprayer, DeVries Manufacturing, Hollandale, MN). Naphthalic anhydride (NA) was dissolved in 3 ml of acetone and then added to the water carrier. Irrigation was withheld for 18 h after treatment application. Herbicides were applied on August 16 and 30, 2012 for experimental runs A and B, respectively. Glasshouse air temperatures averaged 24 C and ranged from 18 to 29 C for the duration of each experimental run.

CBG injury was evaluated 1, 2, and 3 WAT on a 0 (no injury) to 100% (complete leaf tissue necrosis) scale. Only CBG injury data collected 2 WAT will be presented, as treatment responses were most apparent at this time. CBG injury was also quantified via measurements of turfgrass cover via digital image analysis (DIA) using methods modified from Richardson et al. (2001) and Karcher and Richardson (2003). CBG cone-tainers were placed into a custom-constructed light box with a blue background and digitally photographed with a Canon G12 (Canon Inc., Melville, NY) camera. Sigma Scan Pro Software (v. 5.0, SPSS. Inc., Chicago, IL) was used to determine the number of green pixels in each image. Green pixels were defined as those having a hue between 60 and 102 and saturation between 0 and 100% to exclude herbicide-injured leaves (defined as hue < 60; 103 to 134) and the blue background (hue range 135 to 153) from selection. Additionally, clipping yield was quantified 3 WAT to measure how pinoxaden affected the vigor of CBG. Henry and Hart (2004) also used this technique to assess CBG and velvet bentgrass injury from the ACCase inhibitor fenoxaprop. Scissors were used to remove foliage above 1.3 cm. Harvested tissue was then placed in a drying oven at 80 C for 48 h and weighed. Clipping yields were transformed to a percentage of the nontreated control.

Experiment 2: Evaluate Safener Efficacy at Reduced Rates. Additional glasshouse research evaluated the efficacy of safeners identified as efficacious in experiment 1. Cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were evaluated at 450, 225, 90, 68, 45, 23, or 0 g ha^{-1} in combination with pinoxaden at 90 g ha^{-1}. CBG was treated with safeners 30 min before pinoxaden application. Comparisons were made with a nontreated control. Treatments were arranged in a three (safener)-by-seven (safener rate) completely randomized factorial design with five replications. Plant culture, treatment application, and data collection were conducted in the same manner as experiment 1. Treatments for experimental runs A and B were applied on March 19 and October 11, 2013. Glasshouse air temperatures averaged 23 C and ranged from 16 to 32 C during run A. In run B, air temperatures averaged 19 C and reached a maximum of 28 C, but a problem with the glasshouse heating system resulted in air temperatures as low as 7 C for 4 to 6 h each night from 9 to 16 d after treatment.

Experiment 3: Comparing CBG, Roughstalk Bluegrass, and Perennial Ryegrass Responses. On the basis of results of experiment 2, glasshouse research was conducted to evaluate the response of CBG, roughstalk bluegrass, and perennial ryegrass to pinoxaden alone and in combination with herbicide safeners.

CBG, roughstalk bluegrass, and a perennial ryegrass blend (33% Black Cat II, 33% Apple GL, 33% Home Run; Corbin Turf and Ornamental Supply, Greenville, SC) were seeded to separate 10-cm-diam pots filled with growth media described previously. After grass emergence, pots were hand thinned to contain five plants each. Plants were maintained at a 2.5-cm height for 6 mo using mechanical shears. Plants were fertilized with the complete fertilizer described earlier at 49 kg N ha^{-1} 1 wk after germination, and also 4 and 2 wk before treatment. Plants were also fertilized with complete fertilizer at 12 kg N ha^{-1} 24 h before treatment and every 10 d after treatment. Plants were clipped to a 2.5-cm height with scissors 24 h before treatment and then mowing was suspended. Roughstalk bluegrass, perennial ryegrass, and CBG averaged 9, 5, and 10 tillers per plant, respectively, at application.

CBG, roughstalk bluegrass, and perennial ryegrass were treated with pinoxaden (90 g ha^{-1}) alone or in combination with cloquintocet-mexyl, mefenpyr-diethyl, or fenchlorazole-ethyl at 68 or 450 g ha^{-1}, forming a three (safener)-by-two (safener rate) completely randomized factorial design with four replications. Nontreated controls were included for comparison. Safeners were applied 30 min before pinoxaden application. Treatments for runs A and B were applied on May 7 and 14, 2014, respectively, and experiments were conducted in adjacent greenhouse bays. Although a problem with the air temperature monitoring system prevented measurement during 11 consecutive days of run A and 6 d of run B, glasshouse air temperatures were identical for both runs on days where measurement occurred. Temperatures averaged 26 C and ranged from 18 to 31 C.

CBG injury as well as perennial ryegrass and roughstalk bluegrass control were evaluated 2 and 4 WAT as described previously. Clipping yields were collected 2 and 4 WAT as described previously and transformed to a percentage of the nontreated control (no herbicide or safener). At 4 WAT, the number of surviving perennial ryegrass tillers was counted in each pot. Surviving tillers were defined as those that had green leaf shoots that had grown above the rest of the canopy.

Statistical Analysis. Model assumptions were tested through residual analysis (Shapiro–Wilk statistic) in SAS (Statistical Analysis Software, Inc., Cary, NC). ANOVA, mean separations, and contrasts were conducted in SAS with main effects and all possible interactions tested using the appropriate expected mean-square values as described by McIntosh (1983). Perennial ryegrass clipping yield data collected 4 WAT were subjected to an arcsine square-root transformation. However, nontrans-

Table 2. Creeping bentgrass injury and clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2013. Pinoxaden was applied at 90 g ha^{-1} to all plants except for the nontreated control. Safeners were applied at 450 g ha^{-1} 30 min before pinoxaden. Creeping bentgrass visual injury was evaluated 2 wk after treatment (WAT) on a 0 (no injury) to 100% (complete control) scale. Clippings were collected 3 WAT, dried, weighed, and transformed to a percentage of the nontreated control.

Safener	Injury	Clipping yield
	%	% of nontreated
Naphthalic anhydride[a]	8	95
Isoxadifen-ethyl	7	90
Benoxacor	8	75
Cloquintocet-mexyl	2	106
Fenchlorazole-ethyl	2	120
Mefenpyr-diethyl	3	118
None	9	70
$LSD_{0.05}$[b]	5	27

[a] All treatments were applied with non-ionic surfactant at 0.25% v/v.

[b] Abbreviations: LSD, Fisher's protected LSD test ($\alpha \leq 0.05$)

formed data are presented for clarity. No other data were transformed. Fisher's protected LSD ($P \leq 0.05$) was used to separate means for experiments 1 and 2. For experiment 3, contrasts or paired *t* tests were used to determine if responses observed on safener plus pinoxaden-treated plants differed ($\alpha \leq 0.05$) from pinoxaden-treated plants.

Results and Discussion

Experiment 1: Identify Candidate Safeners. The effect of safener was significant for both CBG visual injury and clipping yield, but not DIA. However, DIA and visual injury were correlated ($r = -0.71$; $P < 0.0001$; data not presented). Safeners applied alone did not cause CBG injury or clipping yield reduction and therefore were not included in the statistical analyses. The nontreated control yielded a mean clipping weight of 162 mg at 3 WAT (data not presented).

Pinoxaden alone caused 9% CBG injury and reduced clipping yield to 70% of the nontreated control at 2 WAT (Table 2). The safeners cloquintocet-mexyl, mefenpyr-diethyl, and fenchlorazole-ethyl reduced pinoxaden injury to ≤ 3%. These safeners also eliminated clipping yield reductions caused by pinoxaden. The safeners NA, isoxadifen-ethyl, and benoxacor had no effect on

Table 3. Creeping bentgrass injury and clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2013. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were applied 30 min before pinoxaden (90 g ha^{-1}) application at 450, 225, 90, 68, 45, or 23 g ha^{-1}. Pinoxaden was applied to all plants except for the nontreated control. Creeping bentgrass visual injury was evaluated at 2 wk after treatment (WAT) on a 0 (no injury) to 100% (complete control) scale. Clippings were collected 3 WAT, dried, weighed, and transformed to a percentage of the nontreated control. Data are combined across safener rates.

	Injury		Clipping yield	
Safener	Run A	Run B	Run A	Run B
	——%——		—% of nontreated—	
Cloquintocet-mexyl[a]	9 b[b]	4 b	47 b	61 bc
Fenchlorazole-ethyl	8 b	5 b	51 b	67 b
Mefenpyr-diethyl	6 c	2 c	62 a	78 a
None	15 a	8 a	26 c	56 c

[a] All treatments were applied with nonionic surfactant at 0.25% v/v.

[b] Within each column, means followed by the same letter are not statistically different by Fisher's protected LSD test ($\alpha \leq 0.05$).

CBG injury or clipping yield reductions caused by pinoxaden. These data indicate that cloquintocet-mexyl, mefenpyr-diethyl, and fenchlorazole-ethyl can increase CBG tolerance to pinoxaden and warrant further investigation to determine optimal application rates.

Experiment 2: Evaluate Safener Efficacy at Reduced Rates. Safener rate-by-experimental run interactions were significant; therefore, runs will be presented separately. Safener rate-by-safener interactions were not significant in either experimental run; therefore data will be presented across safener rates and safeners where appropriate. DIA and visual injury were correlated in run A ($r = -0.78$; $P < 0.0001$) and run B ($r = -0.61$; $P < 0.0001$). The nontreated control yielded a mean clipping weight of 186 and 141 mg in run A and run B respectively (data not presented).

Herbicide Safener Efficacy (Combined across Safener Rates). In run A, mefenpyr-diethyl reduced CBG injury from pinoxaden from 15 to 6%, more than cloquintocet-mexyl or fenchlorazole-ethyl (Table 3). Similarly, clipping yield of CBG treated with pinoxaden and mefenpyr-diethyl measured 62% of the nontreated, higher than pinoxaden applied with cloquintocet-mexyl (47%) or fenchlorazole-ethyl

Table 4. Creeping bentgrass injury and clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2013. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were applied at 450, 225, 90, 68, 45, 23, or 0 g ha^{-1} 30 min before pinoxaden (90 g ha^{-1}) application. Pinoxaden was applied to all plants except for the nontreated control. Creeping bentgrass visual injury was evaluated at 2 wk after treatment (WAT) on a 0 (no injury) to 100% (complete control) scale. Clippings were collected 3 WAT, dried, weighed, and transformed to a percentage of the nontreated control. Data are combined across herbicide safeners.

	Injury		Clipping yield	
Safener rate	Run A	Run B	Run A	Run B
g ha^{-1}	———%———		—% of nontreated—	
450[a]	2 d[b]	1 d	70 a	77 a
225	6 c	4 bcd	57 b	68 ab
90	7 c	4 bcd	59 b	66 ab
68	10 b	2 dc	51 bc	65 ab
45	9 b	5 abc	46 cd	70 ab
23	11 b	7 ab	40 d	66 ab
0	15 a	8 a	26 e	56 b

[a] All treatments were applied with nonionic surfactant at 0.25% v/v.

[b] Within each column, means followed by the same letter are not statistically different by Fisher's protected LSD test ($\alpha \leq 0.05$).

(51%). In run B, pinoxaden applied alone only caused 8% injury and and reduced clipping yield to 56% of the nontreated; however, mefenpyr-diethyl was still the most effective safener as it reduced injury to 2% and clipping yields to only 78% of the nontreated. Although mefenpyr-diethyl was the most effective safener, these responses indicate that all safeners can reduce CBG injury from pinoxaden.

Herbicide Safener Rates (Combined across Safeners). The highest safener rate (450 g ha^{-1}) reduced CBG injury more than any other rate in run A; this rate reduced CBG injury from 15 to 2% in run A and 8 to 1% in run B (Table 4). Compared with pinoxaden applied alone, all safener rates reduced CBG injury in run A, but safener application at $\geq$ 68 g ha^{-1} was required to reduce CBG injury in run B. Safener application at 450 g ha^{-1} was required to increase clipping yield in run B compared with pinoxaden alone. It is not clear what caused the variation between experiments. Air temperatures were slightly lower during run B, but there may be other factors that caused this variation. In field experiments anecdotal observations suggest that pinoxaden injury is usually higher when air temperatures are lower (nonpublished data). However, in this experiment, injury from pinoxaden applied without a safener was higher and clipping yield reductions were lower in run A when air temperatures were higher.

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

Experiment 3: Comparing CBG, Roughstalk Bluegrass, and Perennial Ryegrass Responses. CBG and perennial ryegrass response data were combined across experimental runs. Main-effect interactions with experimental run were detected in roughstalk bluegrass data; therefore, data from each experimental run will be presented separately. Main effects, but not their interactions, were significant. Thus, only main effects will be presented for CBG and perennial ryegrass responses as well as roughstalk bluegrass clipping yield. Safener-by-safener rate interactions were detected in roughstalk bluegrass control data and will be presented.

CBG Responses. In run A, the nontreated control yielded a mean clipping weight of 237 and 214 mg at 2 and 4 WAT, respectively (data not presented). In run B, the nontreated control yielded a mean clipping weight of 201 and 269 mg at 2 and 4 WAT, respectively (data not presented).

At 2 WAT, all safeners and safener rates increased CBG clipping yield and reduced injury compared

Table 5. Creeping bentgrass and perennial ryegrass clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were applied at high or low rates (450 or 68 g ha^{-1}, respectively) 30 min before pinoxaden (90 g ha^{-1}) application. Pinoxaden was applied to all plants except for the nontreated control (NTC). Clippings were collected 2 and 4 wk after treatment (WAT), dried, weighed, and transformed to a percentage of the NTC. Contrasts were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. Data are combined across two experimental runs.

	Creeping bentgrass				Perennial ryegrass			
	2 WAT		4 WAT		2 WAT		4 WAT	
	% of NTC	Contrast	% of NTC	Contrast	% of NTC	Contrast	% of NTC	Contrast
Safener								
Cloquintocet-mexyl[a]	69 a[b]	***[c]	123 a	NS	27 a	NS	12 a	NS
Fenchlorazole-ethyl	56 b	**	138 a	NS	30 a	NS	5 ab	NS
Mefenpyr-diethyl	75 a	***	129 a	NS	30 a	NS	3 b	NS
None	32	—	133	—	31	—	6	—
Safener rate								
High	76 a	***	126 a	NS	26 a	NS	6 a	NS
Low	57 b	**	134 a	NS	32 a	NS	8 a	NS

[a] All treatments were applied with nonionic surfactant at 0.25% v/v.
[b] Within each column, means followed by the same letter are not statistically different by Fisher's protected LSD test ($\alpha \leq 0.05$).
[c] **, ***, significant when $\alpha \leq 0.01$ and 0.001, respectively.

with plants treated with pinoxaden only (Tables 5 and 6). Cloquintocet-mexyl and mefenpyr-diethyl reduced CBG injury more than fenchlorazole-ethyl. Pinoxaden alone reduced CBG clipping yield to 32% of the nontreated control, but application in conjunction with either cloquintocet-mexyl or mefenpyr-diethyl increased clipping yield to 69 and 75% of the nontreated control. CBG injury from pinoxaden alone measured 25%, but was reduced to ≤ 5% by cloquintocet-mexyl and mefenpyr-diethyl and 11% by fenchlorazole-ethyl. The high safener rate (450 g ha^{-1}) reduced injury and provided greater clipping yield than the low (68 g ha^{-1}) rate. By 4 WAT, CBG had recovered from pinoxaden application and clipping yields were similar for all safener treatments and not different

Table 6. Creeping bentgrass injury and perennial ryegrass control after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were applied at high or low rates (450 or 68 g ha^{-1}, respectively) 30 min before pinoxaden (90 g ha^{-1}) application. Pinoxaden was applied to all plants except for the nontreated control. Control was evaluated visually on a 0 (no injury or control) to 100% (complete control) scale 2 and 4 wk after treatment (WAT). Contrasts were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. Data were combined across two experimental runs.

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

[a] All treatments were applied with nonionic surfactant at 0.25% v/v.
[b] Within each column, means by the same letter are not statistically different by Fisher's protected LSD test ($\alpha \leq 0.05$).
[c] *, **, ***, significant when $\alpha \leq 0.05$, 0.01, and 0.001, respectively.

Table 7. Roughstalk bluegrass control after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were applied at high or low rates (450 or 68 g ha^{-1}, respectively) 30 min before pinoxaden (90 g ha^{-1}) application. Pinoxaden was applied to all plants except for the nontreated control. Control was evaluated visually on a 0 (no control) to 100% (complete control) scale 2 wk after treatment (WAT). *t* tests were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. Data from two experimental runs are presented separately.

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

[a] All treatments were applied with nonionic surfactant at 0.25% v/v.

[b] Within each column, means followed by the same letter are not statistically different by Fisher's protected LSD test ($\alpha \leq 0.05$).

[c] *, **, ***, significant when $\alpha \leq 0.05$, 0.01, and 0.001, respectively.

from CBG treated with pinoxaden only. CBG injury was < 5% for all treatments at 4 WAT.

Perennial Ryegrass Responses. In run A, the nontreated control yielded a mean clipping weight of 252 and 208 mg at 2 and 4 WAT, respectively (data not presented). In run B, the nontreated control yielded a mean clipping weight of 291 and 256 mg at 2 and 4 WAT, respectively (data not presented).

Neither safener treatment nor safener rate affected perennial ryegrass clipping yield or reduced control compared with pinoxaden alone at 2 or 4 WAT (Tables 5 and 6). Clipping yield, which was used to assess plant vigor, measured between 3 and 12% of the nontreated control, with perennial ryegrass control ranging from 83 to 95% at 4 WAT. Compared with pinoxaden plus cloquintocet-mexyl, perennial ryegrass control from pinoxaden plus mefenpyr-diethyl was greater and clipping yield was lower at 4 WAT. Perennial ryegrass treated with pinoxaden and cloquintocet-mexyl also averaged seven surviving tillers per pot, compared with only one in mefenpyr-diethyl-treated pots at 4 WAT (data not presented). Compared with perennial ryegrass treated with pinoxaden only, safeners did not affect the number of surviving tillers at 4 WAT (data not presented).

Roughstalk Bluegrass Responses. In run A, the nontreated control yielded a mean clipping weight of 189 and 174 mg at 2 and 4 WAT, respectively (data not presented). In run B, the nontreated control yielded a mean clipping weight of 264 and 252 mg at 2 and 4 WAT, respectively (data not presented).

Mefenpyr-diethyl and cloquintocet-mexyl reduced roughstalk bluegrass control and increased clipping yields at most evaluations compared with pinoxaden alone (Tables 7 and 8). Pinoxaden alone provided 41 and 80% roughstalk bluegrass control at 4 WAT in runs A and B, respectively; cloquintocet-mexyl and mefenpyr-diethyl decreased control to < 10% in run A and < 65% in run B. Clipping yields of the nontreated control were greater in run B. Greater pinoxaden efficacy and response to herbicide safeners in run B could be attributed to a more vigorously growing plant; however, the cause of this increased vigor cannot be explained. Clipping yield responses supported visual observations. The clipping yield of roughstalk bluegrass increased from 21% of the control with pinoxaden alone to 49 and 43% of the control with cloquintocet-mexyl and mefenpyr-diethyl, respectively, at 2 WAT in run A. By 4 WAT in run A, clipping yields of roughstalk bluegrass treated with pinoxaden plus mefenpyr-diethyl or cloquintocet-mexyl were 107 and 113% of the control compared with 69% for pinoxaden alone. On most evaluation dates, fenchlorazole-ethyl did not reduce pinoxaden efficacy or clipping yield compared with pinoxaden alone. High (450 g ha^{-1}) rates of mefenpyr-diethyl

Table 8. Roughstalk bluegrass clipping yield after pinoxaden application in a glasshouse in Knoxville, TN in 2014. The herbicide safeners cloquintocet-mexyl, fenchlorazole-ethyl, and mefenpyr-diethyl were applied at high or low rates (450 or 68 g ha^{-1}, respectively) 30 min before pinoxaden (90 g ha^{-1}) application. Pinoxaden was applied to all plants except for the nontreated control (NTC). Clippings were collected 2 and 4 wk after treatment (WAT), dried, weighed, and transformed to a percentage of the NTC. *t* tests were used to determine if responses of safener-treated plants differed ($\alpha \leq 0.05$) from plants treated with only pinoxaden. Data from two experimental runs are presented separately.

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

[a] All treatments were applied with nonionic surfactant at 0.25% v/v.

[b] Within each column, means followed by the same letter are not statistically different as determined by Fisher's protected LSD test ($\alpha \leq 0.05$).

[c] *, **, ***, significant when $\alpha \leq 0.05$, 0.01, and 0.001, respectively.

and cloquintocet-mexyl decreased control more than low (68 g ha^{-1}) rates at 2 and 4 WAT in run B. Safeners can antagonize weed control in cereal crops as well. Mefenpyr-diethyl and fenchlorazole-ethyl can decrease fenoxaprop-ethyl efficacy against blackgrass, a problematic weed of cereal crops in Europe (Cummins et al. 2009). However, the combination of fenoxaprop-*p*-ethyl and mefenpyr-diethyl is registered for blackgrass control in cereals (Anonymous 2011).

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

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

These experiments only evaluated single herbicide applications in a glasshouse. Although they can be used to assess relative efficacy of different treatments, field research will be needed to understand whether sequential applications are required to control roughstalk bluegrass and perennial ryegrass in CBG. Even if multiple applications are required for complete perennial ryegrass and roughstalk bluegrass control, pinoxaden–safener combinations may be a viable option for turfgrass managers if more research is conducted.

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