Weed Technology 2013 27:596–603

Mesotrione, Topramezone, and Amicarbazone Combinations for Postemergence Annual Bluegrass (*Poa annua*) Control

Matthew T. Elmore, James T. Brosnan, Gregory K. Breeden, and Aaron J. Patton*

Selective annual bluegrass (ABG) control with mesotrione is often inconsistent, and sequential applications might be required for complete control. The complementary nature of *p*-hydroxyphenylpyruvate dioxygenase (HPPD)- and photosystem II (PSII)-inhibiting herbicides is well documented. The HPPD-inhibiting herbicide mesotrione and the PSII-inhibiting herbicide amicarbazone both have efficacy against annual bluegrass and safety on certain cool-season turfgrasses. Topramezone is a HPPD-inhibiting herbicide being investigated for use in turfgrass. Field and greenhouse experiments were conducted to examine single applications of topramezone and mesotrione alone or in combination with amicarbazone for POST ABG control in spring. In greenhouse experiments, the combination of mesotrione (280 g ai ha^{-1}) and amicarbazone (75 g ai ha^{-1}) controlled ABG 70% by 21 d after treatment, > 29% more than either herbicide applied alone; these combinations were determined to be synergistic. Amicarbazone combined with topramezone (14.5 g ai ha^{-1}) provided < 10% ABG control and was not synergistic. When combined with mesotrione, increasing amicarbazone rate to 150 or 255 g ha^{-1} did not increase ABG control compared to 75 g ha^{-1}in field experiments. Combining mesotrione with amicarbazone resulted in a synergistic increase in POST ABG control at 1 and 2 wk after treatment (WAT). When applied alone or in combination with amicarbazone, increasing the mesotrione rate from 90 to 280 g ha^{-1} increased efficacy on ABG in field experiments. The combination of mesotrione at 280 g ha^{-1} and amicarbazone at 75 g ha^{-1} provided > 90% ABG control in field experiments. Future research should focus on sequential applications of mesotrione–amicarbazone combinations for ABG control in locations where ABG is historically more difficult to control.

Nomenclature: Amicarbazone; mesotrione; topramezone; annual bluegrass, *Poa annua* L.
Key words: HPPD, *p*-hydroxyphenylpyruvate dioxygenase, photosystem II, PSII inhibiting, synergism, synergy, tank mix, turfgrass, weed control.

El control selectivo de *Poa annua* (ABG) con mesotrione es frecuentemente inconsistente, y aplicaciones secuenciales podrían ser requeridas para alcanzar un control completo. La naturaleza complementaria de los herbicidas inhibidores de *p*-hydroxyphenylpyruvate dioxygenase (HPPD)- y del fotosistema II (PSII) está bien documentada. El herbicida mesotrione, inhibidor de HPPD, y amicarbazone, inhibidor de PSII, son efectivos contra ABG y son seguros en varios céspedes de clima frío. Topramezone es un herbicida inhibidor de HPPD que está siendo investigado para su uso en céspedes. Se realizaron experimentos de campo y de invernadero para examinar aplicaciones simples de topramezone y de mesotrione solos y en combinación con amicarbazone para el control POST de ABG en la primavera. En los experimentos de invernadero, la combinación de mesotrione (280 g ai ha^{-1}) y amicarbazone (75 g ai ha^{-1}) controlaron ABG 70% a 21 días después del tratamiento, >29% más que cualquiera de estos herbicidas aplicados solos; estas combinaciones fueron consideradas sinérgicas. La combinación de amicarbazone con topramezone (14.5 g ai ha^{-1}) brindó <10% de control de ABG y no fue sinérgica. Cuando se combinó con mesotrione, el incrementar la dosis de amicarbazone a 150 ó 255 g ha^{-1} no incrementó el control de ABG al compararse con 75 g ha^{-1} en los experimentos de campo. El combinar mesotrione con amicarbazone resultó en un aumento sinérgico en el control POST de ABG a 1 y 2 semanas después del tratamiento (WAT). Cuando se aplicó amicarbazone solo o en combinación, el aumentar la dosis de mesotrione de 90 a 280 g ha^{-1} incrementó la eficacia sobre ABG en los experimentos de campo. La combinación de mesotrione a 280 g ha^{-1} con amicarbazone a 75 g ha^{-1} brindó >90% de control de ABG en los experimentos de campo. Investigaciones futuras deberían enfocarse en aplicaciones secuenciales de combinaciones de mesotrione-amicarbazone para el control en sitios donde históricamente ABG ha sido más difícil de controlar.

Annual bluegrass is a winter annual with a bunch-type growth habit, light green color, and prolific seedhead production that can be undesirable in managed turfgrass stands (Beard et al. 1978). Selective POST control of ABG in cool-season turfgrass with herbicides such as ethofumesate and bispyribac-sodium is difficult, often requiring multiple herbicide applications at considerable risk of injury to desirable turfgrass species (Johnson 1983; McDonald et al. 2006; Meyer and Branham 2006). Different ABG ecotypes and biotypes can be affected differently by herbicides, causing inconsistent control (McElroy et al. 2004). Long-term control is also difficult due to the large number of ABG seeds present in the soil. Kaminski and Dernoeden (2007) observed annual germination rates of > 100 ABG plants per 1,000 cm^2 at two locations in Maryland. PRE herbicides can be used to control ABG, but their residual control prohibits overseeding desirable turfgrasses for several weeks after application (Dernoeden 1998).

DOI: 10.1614/WT-D-12-00153.1

* First, second, and third authors: Graduate Research Assistant, Assistant Professor, and Extension Assistant, Department of Plant Sciences, The University of Tennessee, 252 Ellington Plant Sciences Bldg., 2431 Joe Johnson Dr., Knoxville, TN 37996; fourth author: Assistant Professor, Department of Agronomy, Purdue University, West Lafayette, IN 47907; Corresponding author's E-mail: melmore6@utk.edu

Mesotrione and topramezone are *p*-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (Grossmann and Ehrhardt 2007; Mitchell et al. 2001). HPPD inhibition indirectly inhibits production of carotenoid pigments involved with quenching singlet- and triplet-state oxygen (Demmig-Adams et al. 1996; Niyogi 1999). Susceptible plants initially display leaf whitening, which is often followed by necrosis (Lee et al. 1997). Mesotrione is registered for weed control in turfgrass and corn (*Zea mays* L.), whereas topramezone is registered for application in corn (Anonymous 2007, 2011, 2012b).

Reicher et al. (2011) reported POST ABG control from sequential applications of mesotrione at rates ranging from 110 to 280 g ha^{-1}. However, control was inconsistent across years and locations, ranging from 0% after three applications of mesotrione at 280 g ha^{-1} in Illinois in 2005 to $>$ 90% after three applications in Indiana in 2004. Skelton et al. (2012) reported inconsistent ABG control, depending on initial application timing; mesotrione applied biweekly for 6 wk at 186 g ha^{-1} provided 0 and 100% POST ABG control 8 WAT when applied in June and October, respectively. Branham et al. (2010) reported five biweekly applications of mesotrione at 110 g ha^{-1} provided $>$ 95% POST control of ABG in Illinois, but Patton et al. (2011) only observed 40% control in Indiana from the same application regimen. Reports from Indiana and Illinois suggest several sequential fall applications of mesotrione spaced $<$ 2 wk apart provide the best ABG control (Branham et al. 2010; Patton et al. 2011; Reicher et al. 2011; Skelton et al. 2012). Two or three sequential applications of mesotrione at 280 or 175 g ha^{-1}, respectively, applied in March, increased ABG control compared to single applications in Tennessee (Elmore et al. 2012a). These previous reports of ABG control examined mesotrione only, and demonstrated that sequential applications are required for acceptable POST ABG control. Greater control from single applications in early spring (March) applications that occur after the majority of ABG germination would be beneficial. Additionally, there are no published reports of topramezone efficacy against ABG to date.

Amicarbazone is a photosystem II (PSII)-inhibiting herbicide registered for use in corn, sugarcane (*Saccharum officinarum* L.) and turfgrass with efficacy against ABG (Anonymous 2012a; Dayan et al. 2009; McCullough et al. 2010; Senseman 2007). Amicarbazone absorption in ABG occurs through foliage and roots (Perry et al. 2011). McCullough et al. (2010) suggested that amicarbazone has potential to control ABG in cool-season grasses, particularly perennial ryegrass and Kentucky bluegrass (*Poa pratensis* L.), when applied in the spring.

When applied POST, control of broadleaf weeds with mesotrione can be enhanced with the addition of the PSII-inhibitor atrazine (Abendroth et al. 2006; Armel et al. 2005, 2007; Hugie et al. 2008; Sutton et al. 2002; Woodyard et al. 2009). Synergy has been demonstrated with low rates of atrazine and/or mesotrione in atrazine- and triazine-resistant redroot pigweed (*Amaranthus retroflexus* L.) and velvetleaf (*Abutilon theophrasti* Medik.) (Woodyard et al. 2009). Mixtures of mesotrione plus atrazine controlled Canada thistle (*Cirsium arvense* L. Scop.) better than mesotrione alone (Armel et al. 2005). Enhanced control is likely a result of the complementary nature of the HPPD and PSII-inhibiting herbicidal modes of action (Kim et al. 1999). Amicarbazone inhibits PSII electron transfer by binding to the Q_B niche on the D1 protein (Dayan et al. 2009). This indirectly blocks the transfer of energy from excited chlorophyll molecules to the PSII reaction center, generating excited triplet chlorophyll ($^3Chl^*$), which reacts with molecular oxygen to form excited singlet oxygen ($^1O_2^*$) (Fuerst and Norman 1991). This toxic singlet oxygen causes lipid peroxidation and can destroy membrane integrity. Quenching of singlet oxygen and triplet chlorophyll by carotenoid pigments renders them nontoxic and unable to cause lipid peroxidation (Demmig-Adams et al. 1996). The HPPD-inhibiting herbicides topramezone and mesotrione indirectly inhibit carotenoid production in susceptible plants (Grossmann and Ehrhardt 2007; Mitchell et al. 2001). Increased production and decreased quenching of singlet oxygen in plants treated with both HPPD- and PSII-inhibitors is likely the cause of their complementary mode of action.

Research investigating strategies to improve ABG control with mesotrione is warranted, given the variability that has been observed with mesotrione in field studies (Reicher et al. 2011; Skelton et al. 2012). Given that synergy with PSII- and HPPD-inhibiting herbicide tank mixtures is well documented, we hypothesized that combinations of mesotrione or topramezone with amicarbazone would control ABG better than either herbicide applied alone. Thus, the objective of this research was to evaluate ABG control with mesotrione or topramezone applied alone and in combination with amicarbazone.

Materials and Methods

Greenhouse Research. *Study 1—Amicarbazone Mixtures.* Two identical experiments were initiated in 2011 in a greenhouse (Knoxville, TN; 35°57′N, 83°56′W) to evaluate control of ABG with mesotrione and topramezone when applied alone and in combination with amicarbazone. Single tillers of ABG were harvested from the East Tennessee Research and Education Center (Knoxville, TN) and transplanted to 3.8-cm-diam cone-tainers (SC10 super cell cone-tainer; Steuwe and Sons, Tangent, OR) filled with Sequatchie loam soil (fine-loamy, siliceous, semiactive, thermic Humic Hapludult) measuring 6.2 in soil pH and 2.1% in organic matter content. At transplant, 25 kg ha^{-1} nitrogen (N) was applied from 5 mL of a complete fertilizer (20–8.7–16.6; Howard Johnson's Triple Twenty Plus Minors, Milwaukee, WI) solution to each cone-tainer. Plants were maintained at a 2.5 cm height with hand-shears and watered as needed to maximize growth and vigor. Plants were allowed to acclimate for 4 wk and contained 10 to 12 tillers when treatments were applied. Prior to herbicide application, N was applied at 50 kg ha^{-1} because previous research has shown N enhances mesotrione efficacy (Cathcart et al. 2004; Elmore et al. 2012b). Nitrogen was applied to each cone-tainer using 5 mL of solution containing 4.9 g of urea (46-0-0) dissolved in 2 L of water 1 h prior to herbicide application, and plants were clipped for the final time at 2.5 cm.

https://doi.org/10.1614/WT-D-12-00153.1 Published online by Cambridge University Press

Environmental conditions were similar in each experimental run. Air temperatures in the greenhouse ranged from 14 to 29 C in experimental run A and 15 to 29 C in run B; air temperature averaged 21 and 22 C for runs A and B, respectively. Peak 24-h photosynthetically-active radiation (PAR) values averaged 673 and 743 µmol m^{-2} s^{-1} during experimental runs A and B, respectively. Daily PAR flux averaged 8.5 and 10.2 mol m^{-2} d^{-1} during experimental runs A and B, respectively.

Treatments consisted of mesotrione (Tenacity; Syngenta Professional Products, Greensboro, NC) at 280 g ha^{-1} or topramezone (Impact; Amvac Chemical, Los Angeles, CA) at 14.5 g ha^{-1} alone or tank mixed with amicarbazone (Xonerate; Arysta LifeSciences, Cary, NC) at 75 g ha^{-1}. A nontreated control was included for comparison. This arrangement formed a 3 by 2 factorial completely randomized treatment design that was replicated 10 times and repeated in time. All herbicide treatments were applied with a nonionic surfactant (Activator-90; Loveland Products Inc., Loveland, CO) at 0.25% v/v and 440 L ha^{-1} water using a spray chamber (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) equipped with an 8002E flat-fan nozzle (TeeJet, Spraying Systems Co., Wheaton, IL).

ABG control was visually evaluated at 5, 7, 14, and 21 d after treatment (DAT) on a 0 (no control) to 100 % (complete kill) scale. Visual estimates ABG control were similar at 5 and 7 DAT; therefore, observations from 5 DAT are not presented. Chlorophyll fluorescence (F_v/F_m) yield (OS1-FL; Opti-sciences Inc., Hudson, NH) was also assessed using methods of Elmore et al. (2011) at 5 and 7 DAT. Only F_v/F_m data from 7 DAT are presented, because responses were similar at 5 and 7 DAT. Aboveground biomass was harvested from each cone-tainer 21 DAT, dried at 100 C for 24 h, and weighed. To determine the percent biomass reduction, weights of treated plants were compared to the mean of the nontreated control plants within each replication. Because poor ABG control was observed with topramezone applied alone or in combination with amicarbazone, this herbicide was not included in Study 2.

Study 2—Low-Rate Mesotrione Mixtures with Amicarbazone. Two identical experiments were initiated in 2012 in a greenhouse (Knoxville, TN) to evaluate several rates of mesotrione applied alone and in combination with amicarbazone for ABG control. Annual bluegrass seed was collected from the Joseph Valentine Turfgrass Research Center (University Park, PA) in May 2008 from a site maintained as a simulated golf course fairway for > 5 yr until mowing was suspended in April 2008 to promote inflorescence formation and facilitate seed harvest. In December 2011, seeds were planted to 3.8-cm-diam cone-tainers filled with the same Sequatchie silt loam used in Study 1. Three wk after seeding and 4 wk prior to treatment application, 25 and 15 kg ha^{-1} N, respectively, was applied from 5 mL of complete fertilizer (20–8.7–16.6) solution to individual cone-tainers. Plants were maintained in the same manner as in Study 1 and contained two to three tillers when treatments were applied.

Treatments consisted of mesotrione applied at 90, 175, or 280 g ha^{-1} alone or in combination with amicarbazone at 75 g ha^{-1}. Amicarbazone was also applied alone at 75 g ha^{-1}, and a nontreated control was included for comparison. This arrangement formed a 3 by 2 factorial completely randomized treatment design that was replicated 10 times and repeated in time. All herbicide treatments were applied and evaluated in the same manner as Study 1.

Air temperatures in the greenhouse ranged from 14 to 29 C in experimental run A and 16 to 29 C in run B; air temperature averaged 23 C for both experiments. Peak 24-h PAR values averaged 1,081 and 1,202 µmol m^{-2} s^{-1} during experimental runs A and B, respectively. PAR flux averaged 14.2 and 16.4 mol m^{-2} d^{-1} during experimental runs A and B, respectively.

Field Research. Research was conducted in 2011 and 2012 on a dormant 'Tifway' bermudagrass [*Cynodon dactylon* (L.) Pers.] stand overseeded with perennial ryegrass (PRG; 45% 'SR4600′, 32% 'SR4350′, 22% 'Pavillion') at 440 kg ha^{-1} in September 2010 and 2011 and naturally infested with ABG in Rockford, TN (35°49′N, 83°56′W). The site was maintained as a golf course fairway; mowing occurred twice per week with a reel-mower at 1.3-cm height, and the site was irrigated as needed to maximize growth and vigor. ABG cover was estimated to be 20% and 30% when field experiments were initiated in 2011 and 2012, respectively. The soil was a Hamblen (fine-loamy, siliceous, semiactive, thermic Fluvaquentic Eutrudept) silt loam soil with a pH of 6.4 and 2.8% organic matter content.

Mesotrione–Amicarbazone Combinations. Research was conducted at the Rockford, TN location in 2011 and 2012 evaluating the ABG control efficacy of HPPD-inhibiting herbicides tank mixed with amicarbazone. Treatments in 2011 included the factorial combination of two HPPD inhibitors, mesotrione (280 g ha^{-1}) or topramezone (14.5 g ha^{-1}), and three rates (75, 150, and 255 g ha^{-1}, respectively) of amicarbazone. Treatments were applied with the same nonionic surfactant used in greenhouse experiments at 0.25 % v/v and water at 280 L ha^{-1} using a CO_2-pressurized sprayer containing four flat-fan nozzles (TeeJet XR8002VS flat fan spray nozzle; Spraying Systems Co., Roswell, GA). Urea was broadcast to the entire site at 50 kg ha^{-1} N and watered in immediately prior to herbicide treatment. The air temperature measured 8 C and the relative humidity measured 71% when treatments were applied on March 16, 2011 to plots measuring 1.5 by 1.5 m. Plants in the ABG population were estimated to contain between 2 and 10 tillers at application. The experimental design was a randomized complete block with three replications. Comparisons were made to a nontreated control. Bispyribac-sodium (Velocity; Valent U.S.A Corp., Walnut Creek, CA) applied at 80 g ai ha^{-1} was included as a standard.

In 2012, experiments were conducted at the same location in Rockford, TN. In 2012, treatments included the factorial combination of four rates (none, low, medium, and high) of mesotrione (0, 90, 175, and 280 g ha^{-1}, respectively) and four rates (none, low, medium, and high) of amicarbazone (0, 75, 150, and 255 g ha^{-1}, respectively). Because tank mixing topramezone with amicarbazone did not improve control compared to amicarbazone alone, this herbicide was not included. Treatments applied in 2012 contained the same

Table 1. Chlorophyll fluorescence yield (F_v/F_m) of annual bluegrass (ABG; *Poa annua* L.) leaf tissue 7 DAT, POST ABG control 7, 14, and 21 d after treatment (DAT) and aboveground ABG dry biomass 21 DAT with mesotrione (280 g ai ha^{-1}), topramezone (14.5 g ai ha^{-1}), and amicarbazone (75 g ai ha^{-1}) in two greenhouse experiments in Knoxville, TN in 2011.

Treatment	Chlorophyll fluorescence yield 7 DAT	ABG control[a] 7 DAT	ABG control[a] 14 DAT	ABG control[a] 21 DAT	ABG biomass 21 DAT
	F_v/F_m[b]	%	%	%	% reduction
Amicarbazone[c]	0.55	0	4	4	44
Mesotrione	0.22	19	38	44	61
Mesotrione + amicarbazone	0.11	25	72[d]	74[d]	74
Topramezone	0.54	6	2	2	24
Topramezone + amicarbazone	0.44	4	5	9	57
Nontreated control	0.74	0	0	0	0
$LSD_{0.05}$[e]	0.05	5	7	10	22

[a] Control was evaluated visually on a 0 (no control) to 100% (complete kill) scale relative to the nontreated control.

[b] F_v/F_m = Chlorophyll fluorescence yield.

[c] All herbicide treatments were applied with nonionic surfactant (NIS) at 0.25% v/v.

[d] Control determined to be synergistic because observed control was significantly greater than the expected control as determined by the Colby equation (Colby 1967). Significant differences of observed and expected means were determined using a *t* test in SAS ($\alpha \leq 0.05$).

[e] Abbreviation: LSD, Fisher's least significant difference ($\alpha \leq 0.05$).

mesotrione–amicarbazone combinations as 2011, in addition to lower mesotrione rates in combination with amicarbazone. A nontreated control was included for comparison at both locations. Bispyribac-sodium (80 g ha^{-1}) was included as a standard. Herbicide treatments and urea N were applied in the same manner as in 2011 at the Tennessee location on March 8, 2012 to plots measuring 1.5 by 3.0 m. The air temperature measured 17 C and the relative humidity measured 60% when treatments were applied. Plants in the ABG population were estimated to contain between 2 and 10 tillers at application. The experimental design was a randomized complete block with three replications.

In 2011 and 2012, ABG control was visually evaluated at 1, 2, 3, 4, 6, and 8 WAT on a 0 (no control) to 100% (complete kill) scale relative to the nontreated control. Responses from 3, 4, and 6 WAT were similar; only responses from 3 WAT are presented. To quantitatively assess treatment responses, grid counts were conducted 8 WAT using a 100- by 100-cm grid containing 81 squares (10 by 10 cm). The presence or absence of ABG in each square was recorded with means generated using two subsamples per plot. ABG showed signs of natural senescence beginning 6 WAT in both years and was more rapid in 2012 than in 2011. PRG injury was visually evaluated weekly until 3 WAT on a 0 (no injury) to 100% (complete kill) scale in 2011. Injury was not evaluated in 2012 due to low PRG stand density in nontreated plots.

Statistical Analyses. Data from all experiments were subjected to ANOVA in SAS 9.3 (Statistical Analysis Software, Inc., Cary, NC) with main effects and all possible interactions tested using the appropriate expected mean square values as described by McIntosh (1983). Model assumptions were tested through residual analysis (Shapiro–Wilk statistic) in SAS. Perennial ryegrass injury data were arcsine–square-root transformed. Interpretations of transformed data were not different from nontransformed data; therefore nontransformed data are presented. Fisher's protected LSD ($\alpha \leq 0.05$) was used to separate means. Expected values for herbicide interactions were calculated using the Colby equation (Equation 1; Colby 1967):

$$E = (X + Y) - (XY/100) \quad [1]$$

where E is the expected control provided by herbicides mesotrione + amicarbazone, X is the observed control from mesotrione, and Y is the observed control from amicarbazone. The expected and observed values of mesotrione + amicarbazone were subjected to a *t* test to determine whether the means were different. If the observed mean was greater than the expected mean, the combination was deemed synergistic.

Results and Discussion

Greenhouse Research. *Study 1.* No main effect interactions with experimental run were detected; therefore, all data were combined across experimental runs.

All herbicide treatments reduced chlorophyll fluorescence yield (F_v/F_m) compared to the nontreated control 7 DAT (Table 1). Mesotrione reduced $F_v/F_m > 50\%$ compared to the nontreated control. The greatest reductions in F_v/F_m were observed in plants treated with mesotrione + amicarbazone; this treatment reduced $F_v/F_m > 80\%$ compared to the nontreated control. Topramezone and amicarbazone applied alone reduced $F_v/F_m < 40\%$, less than mesotrione-treated plants.

Measurements of F_v/F_m yield supported assessments of ABG control with applied treatments. Topramezone- and amicarbazone-treated plants were controlled < 10% on all rating dates. Combining amicarbazone with mesotrione resulted in a synergistic increase in ABG control at 14 and 21 DAT; control with the combination was > 70% compared to < 45% from mesotrione alone. Topramezone + amicarbazone combinations were not synergistic. This could be a result of low (< 10%) topramezone efficacy observed in the experiments. Evaluating combinations of mesotrione and atrazine on redroot pigweed (*Amaranthus retroflexus* L.), Hugie et al. (2008) demonstrated that with a constant rate of atrazine, synergism was not observed from mesotrione rates

Table 2. POST annual bluegrass (ABG; *Poa annua* L.) control after treatment with mesotrione (280, 175, and 90 g ai ha^{-1}) and amicarbazone (0 or 75 g ai ha^{-1}) in two experiments conducted in a greenhouse in Knoxville, TN in 2012.

Treatment		Chlorophyll fluorescence yield		ABG control[a]					
		Run A	Run B	Run A			Run B		
Mesotrione[b]	Amicarbazone	7 DAT[c]		7 DAT	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT
g ha^{-1}		F_v/F_m[d]		%					
90	0	0.48	0.49	14	9	6	20	6	8
175	0	0.27	0.43	32	25	10	22	13	13
280	0	0.17	0.29	42	32	15	35	19	21
90	75	0.07	0.03	50[e]	75[e]	55[e]	46[e]	77[e]	88[e]
175	75	0.02	0.04	63[e]	92[e]	85[e]	51[e]	81[e]	85[e]
280	75	0.01	0.03	59[e]	92[e]	91[e]	52[e]	83[e]	93[e]
0	75	0.30	0.22	6	35	29	0	42	57
0	0	0.69	0.68	0	0	0	0	0	0
$LSD_{0.05}$		0.05	0.07	5	7	8	6	6	6

[a] Control was evaluated visually on a 0 (no control) to 100% (complete kill) scale relative to the nontreated control.

[b] All herbicide treatments were applied with nonionic surfactant (NIS) at 0.25% v/v.

[c] Abbreviations: DAT, days after treatment; LSD, Fisher's least significant difference ($\alpha \leq 0.05$).

[d] F_v/F_m = Chlorophyll fluorescence yield.

[e] Control determined to be synergistic because observed control was significantly greater than the expected control as determined by the Colby equation (Colby 1967). Significant differences of observed and expected means were determined using a *t* test in SAS ($\alpha \leq 0.05$).

below 56 g ha^{-1}. They suggested that a certain threshold of mesotrione is required to provide a synergistic response. It is likely that this threshold of HPPD-inhibition was not exceeded after topramezone application.

All herbicide treatments reduced aboveground biomass compared to the nontreated control (Table 1). Biomass of mesotrione + amicarbazone-treated plants was reduced by 74%, but reductions were not significantly different from mesotrione applied alone. Amicarbazone applied alone reduced biomass by 44%. Topramezone treatment reduced biomass by 24%. Although the addition of amicarbazone to topramezone increased biomass reduction to 57%, this response was not significantly different from that of amicarbazone alone. When control was determined visually, plants treated with amicarbazone alone or in combination with topramezone was low (< 10%), but reductions in biomass ranged were higher, ranging from 24 to 57%. We are unsure of the reason for this disparity.

These results demonstrate that mesotrione injures ABG at 280 g ha^{-1} and can be synergized by the addition of amicarbazone at 75 g ha^{-1}. This is similar to previous reports of enhanced efficacy and synergism from combinations of mesotrione and atrazine (Abendroth et al. 2006; Armel et al. 2005, 2007; Hugie et al. 2008; Woodyard et al. 2009). Topramezone efficacy against ABG was poor and did not enhance amicarbazone efficacy. When applied alone at 75 g ha^{-1}, amicarbazone displayed little efficacy against ABG.

Study 2. Main-effect interactions with experimental run were detected; therefore, data from each experimental run are discussed separately. Amicarbazone-by-mesotrione interactions detected in chlorophyll fluorescence yield and visual estimates of control are presented. This same interaction was not detected in aboveground biomass data; only main effects of mesotrione and amicarbazone were significant. This was caused by amicarbazone causing similar reductions in biomass across all rates of mesotrione.

Similar to responses observed in Study 1 (Table 1), the lowest F_v/F_m values were observed with mesotrione + amicarbazone in both experimental runs (Table 2). Mesotrione rate did not affect F_v/F_m of plants treated with mesotrione + amicarbazone combinations. Amicarbazone applied alone and all rates of mesotrione reduced F_v/F_m compared to the nontreated control on all observation dates. When mesotrione was applied alone, the 280 g ha^{-1} rate reduced F_v/F_m more than the 90 g ha^{-1} rate 7 DAT in both experimental runs.

Applied alone, mesotrione and amicarbazone controlled ABG < 60% on all rating dates in each experimental run. Control from mesotrione–amicarbazone combinations was synergistic and greater than all other treatments 7, 14, and 21 DAT in both experimental runs. In combination with amicarbazone, 175 and 280 g ha^{-1} of mesotrione provided the greatest (85 and 91%, respectively) control of ABG in run A at 21 DAT; all rates of mesotrione applied with amicarbazone controlled ABG similarly in run B (85 to 93%) as well. Supporting visual observations, biomass was lowest in plants treated with amicarbazone and the 280 g ha^{-1} and 175 g ha^{-1} rates of mesotrione (data not presented).

Data from greenhouse Studies 1 and 2 demonstrate that tank mixtures of mesotrione and amicarbazone often are synergistic for ABG control. Efficacy of topramezone against ABG was minimal, even when tank mixed with amicarbazone. Additionally, reducing the mesotrione rate to as low as 175 g ha^{-1} did not reduce ABG control when amicarbazone was included in the tank mixture, except at 21 DAT in Run B.

Field Research. Interactions of the HPPD-inhibitors with amicarbazone rate were significant on most dates; therefore, the interaction is presented for 2011 data. Although several treatments in the 2011 experiment overlap with 2012 treatments, 2012 data are presented separately for clarity. Synergistic interactions were detected in 2011 experiments.

Table 3. POST annual bluegrass (ABG; *Poa annua* L.) control after treatment with amicarbazone (75, 150, or 255 g ai ha^{-1}) tank mixed with or without mesotrione (280 g ai ha^{-1}) in field experiments conducted in Rockford, TN in 2011.

Herbicide[a]		ABG control[b]				Plant count [c]
Amicarbazone	Mesotrione	1 WAT[d]	2 WAT	3 WAT	8 WAT	8 WAT
g ha^{-1}		%				% reduction
75	0	10	5	47	58	14
150	0	12	12	40	78	43
255	0	10	37	92	96	85
75	280	50	80[e]	93	97	87
150	280	52	87[e]	95	98	97
255	280	58[e]	85	93	99	99
0	280	40	38	50	78	60
$LSD_{0.05}$		10	25	30	17	25

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

[b] Control was evaluated visually on a 0 (no control) to 100% (complete kill) scale relative to the nontreated control.

[c] The presence or absence of annual bluegrass in a grid containing 81 10- by 10-cm squares was noted. Two grid counts were taken in each plot, averaged, and compared to the nontreated control to determine the percent reduction for each treatment. Nontreated plots averaged 77 squares containing annual bluegrass.

[d] Abbreviations: WAT, weeks after treatment; LSD, Fisher's least significant difference ($\alpha \leq 0.05$).

[e] Control determined to be synergistic because observed control was significantly greater than the expected control as determined by the Colby equation (Colby 1967. Significant differences of observed and expected means were determined using a *t* test in SAS ($\alpha \leq 0.05$).

In 2011, ABG control with topramezone applied alone was < 15% on all rating dates and topramezone + amicarbazone controlled ABG similar to amicarbazone alone (data not presented). Mesotrione in combination with all rates of amicarbazone controlled ABG ≥ 50% at 1 WAT, greater than that provided by either herbicide applied alone (Table 3). The combination of mesotrione + amicarbazone at 255 g ha^{-1} was synergistic 1 WAT. By 2 WAT, all mesotrione–amicarbazone combinations provided ≥ 80% ABG control; applied alone these herbicides controlled ABG < 40% at 2 WAT. Combinations of mesotrione and amicarbazone at 75 and 150 g ha^{-1} were synergistic 2 WAT. From 3 to 8 WAT, all mesotrione–amicarbazone combinations provided > 90% ABG control, which was more than that provided by mesotrione or the 75 g ha^{-1} rate of amicarbazone applied alone. ABG control from all mesotrione–amicarbazone combinations were similar 2 to 8 WAT. The highest rate of amicarbazone (255 g ha^{-1}) controlled ABG similar to all mesotrione–amicarbazone combinations 3 to 8 WAT as well. Data from plant counts support visual observations. Bispyribac-sodium controlled ABG > 90% 8 WAT (data not presented).

In 2012, ABG control varied due to the main effects of mesotrione rate and amicarbazone rate 2 to 8 WAT in Tennessee, but mesotrione-by-amicarbazone interactions were not significant; therefore, main effects are presented. In Tennessee, ABG control was similar with all rates of amicarbazone on every rating date (Table 4). Amicarbazone provided greater ABG control than treatments not containing amicarbazone (i.e., mesotrione-only treatments) 2 to 8 WAT.

Table 4. POST annual bluegrass (ABG; *Poa annua* L.) control following applications of amicarbazone (0, 75, 150, or 255 g ai ha^{-1}) and mesotrione (0, 90, 175, or 280 g ai ha^{-1}) in field experiments conducted in Rockford, TN in 2012. Mesotrione and amicarbazone were applied alone or in complete factorial combination with each other. Only main effects are presented because main effect interactions were not significant; thus, the effect of amicarbazone rate is presented as an average across all mesotrione rates and the effect of mesotrione rate is presented as an average across all amicarbazone rates.

Herbicide	Rate	ABG control[a]			Grid count[b]
		2 WAT[c]	3 WAT	8 WAT	8 WAT
	g ha^{-1}	%			% reduction
Amicarbazone[d]	0	10	26	17	8
	75	40	60	59	38
	150	42	59	65	45
	255	42	70	60	39
Mesotrione	0	26	33	29	17
	90	15	45	43	32
	175	37	57	53	25
	280	53	74	68	52
$LSD_{0.05}$		17	15	17	7

[a] Control was evaluated visually on a 0 (no control) to 100% (complete kill) scale relative to the nontreated control.

[b] The presence or absence of annual bluegrass in a grid containing 81 10- by 10-cm squares was noted. Two grid counts were taken in each plot, averaged, and compared to the nontreated control to determine the percent reduction for each treatment. Nontreated plots averaged 15 squares containing annual bluegrass.

[c] Abbreviations: WAT, weeks after treatment; LSD, Fisher's least significant difference ($\alpha \leq 0.05$).

[d] All herbicide treatments were applied with nonionic surfactant (NIS) at 0.25% v/v.

https://doi.org/10.1614/WT-D-12-00153.1 Published online by Cambridge University Press

Mesotrione rate did affect ABG control on most rating dates in Tennessee. Mesotrione applied at 280 g ha^{-1} provided greater ABG control than mesotrione applied at 90 g ha^{-1} from 2 to 8 WAT and greater control than 90 and 175 g ha^{-1} at 3 WAT. Grid counts support visual estimates of control. Similar to 2011, bispyribac-sodium controlled ABG $> 90\%$ at 8 WAT (data not presented).

Compared to greenhouse experiments, synergy was less prevalent in field experiments. Synergism was detected only at 1 or 2 WAT for mesotrione + amicarbazone combinations in 2011 field experiments; no synergism was detected in 2012 field experiments. By 3 WAT, control from amicarbazone and mesotrione alone was greater in field experiments than greenhouse experiments, reducing the potential for synergy. Lower levels of ultraviolet (UV) radiation, particularly that between 280 and 320 nm (UV-B) might have been lower in the greenhouse environment and reduced control from herbicides applied alone. The amount of UV radiation was not measured in greenhouse experiments, but window glass prevents transmission of nearly all UV-B radiation (Committee on Environmental Health 1999). Carotenoid pigments and other antioxidants function to protect the plant from oxidative damage caused by UV-B radiation (Joshi et al. 2011; Pallett and Young 1993; Strid et al. 1994; Zhang et al. 2005). Additionally, exposure to UV-B radiation can degrade the D1 protein and reduce thylakoid membrane integrity, reducing the light-driven, pH-dependent conversion of the xanthophyll cycle carotenoid violaxanthin (via violaxanthin de-epoxidase) to the more photoprotective zeaxanthin (Pfündel et al. 1992; Strid et al. 1994). Damage caused by UV-B radiation to carotenoid pigments and reduction in thylakoid membrane integrity might complement carotenoid biosynthesis inhibition and increases in oxidative damage caused by HPPD- and PSII-inhibiting herbicides, thus increasing control observed in field experiments.

In 2011, PRG injury was highest 2 WAT in Tennessee (data not presented). Amicarbazone applied alone did not injure PRG on any rating date. Mesotrione applied alone injured PRG 18%. This level of injury was not unexpected because the 280 g ha^{-1} rate of mesotrione exceeds current labeling for use on PRG (Anonymous 2011). The addition of amicarbazone at 255 g ha^{-1} to mesotrione increased injury to 28%. Although these treatments exceed the maximum-labeled use rate for mesotrione (175 g ha^{-1}), responses indicate that PRG injury from mesotrione is exacerbated by amicarbazone applications.

Responses observed in this greenhouse and field research suggest that combinations of mesotrione and amicarbazone can provide greater control of ABG when tank mixed. Field experiments in Tennessee indicated that increasing the mesotrione rate to 280 g ha^{-1} is beneficial for ABG control; however, there is no benefit to increasing the amicarbazone rate > 75 g ha^{-1} in mixtures with mesotrione. Future research should evaluate mesotrione at 280 g ha^{-1} in combination with amicarbazone applied at less than 75 g ha^{-1} to determine the effect on ABG control. Fall applications of mesotrione and amicarbazone have been reported to provide better POST ABG control than spring applications (McCullough et al. 2010; Skelton et al. 2012). Future research should focus on sequential applications of mesotrione–amicarbazone combinations applied in the fall using programs similar to those developed by Skelton et al. (2012) in locations where ABG is historically more difficult to control. The effect of fall and spring applications on PRG and Kentucky bluegrass injury also needs to be evaluated, because fall applications of amicarbazone can be more injurious to desirable turfgrasses (Anonymous 2012a; McCullough et al. 2010).

Acknowledgments

The authors would like to thank Jake Huffer, Daniel Farnsworth, and Shane Breeden for their assistance in conducting these experiments, and Jeff Borger for supplying the seed used in greenhouse experiments. Additionally, the authors would like to thank Donald Lequire of Egwani Farms for maintaining the experiment site. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of Tennessee Institute of Agriculture or Purdue University.

Literature Cited

Abendroth, J. A., A. R. Martin, and F. W. Roeth. 2006. Plant response to combinations of mesotrione and photosystem II inhibitors. Weed Technol. 20:267–274.

Anonymous. 2007. Impact herbicide label. Amvac. Los Angeles, CA. 4 p.

Anonymous. 2011. Tenacity herbicide label. Greensboro, NC: Syngenta Crop Protection, Inc. 16 p.

Anonymous. 2012a. Xonerate herbicide label. Cary, NC: Arysta LifeScience. 34 p.

Anonymous. 2012b. Callisto herbicide label. Greensboro, NC: Syngenta Crop Protection, Inc. 32 p.

Armel, G. R., G. J. Hall, H. P. Wilson, and N. Cullen. 2005. Mesotrione plus atrazine mixtures for control of Canada thistle (*Cirsium arvense*). Weed Sci. 53:202–211.

Armel, G. R., P. L. Rardon, M. C. McCormick, and N. M. Ferry. 2007. Differential response of several carotenoid biosynthesis inhibitors in mixture with atrazine. Weed Technol. 21:947–953.

Beard, J. B., P. E. Rieke, A. J. Turgeon, and J. M. Vargas, Jr. 1978. Annual bluegrass (*Poa annua* L.) description, adaptation, culture, and control. Agricultural Experiment Station Research Report 352. East Lansing, MI: Michigan State University. 32 p.

Branham, B., B. Sharp, and J. Skelton. 2010. Mesotrione provides postemergence control of *Poa annua* L. Paper 77-13 in ASA, CSSA, and SSSA Annual Meetings. Long Beach, CA: Crop Science Society of America.

Cathcart, R. J., K. Chandler, and C. J. Swanton. 2004. Fertilizer nitrogen rate and the response of weeds to herbicides. Weed Sci. 52:291–296.

Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15:20–22.

Committee on Environmental Health. 1999. Ultraviolet light: A hazard to children. Pediatrics. 104:328–333.

Dayan, F. E., M.L.B. Trindale, and E. D. Velini. 2009. Amicarbazone, a new photosystem II inhibitor. Weed Sci. 57:579–583.

Demmig-Adams, B., A. M. Gilmore, and W. W. Adams, III. 1996. Carotenoids 3: in vivo function of carotenoids in higher plants. FASEB J. 10:403–412.

Dernoeden, P. H. 1998. Use of prodiamine as a preemergence herbicide to control annual bluegrass in Kentucky bluegrass. HortScience 33:845–846.

Elmore, M. T., J. T. Brosnan, and G. K. Breeden. 2012a. Strategies for controlling annual bluegrass (*Poa annua* L.) using mesotrione. Bioforsk Fokus 7:121–122.

Elmore, M. T., J. T. Brosnan, D. A. Kopsell, and G. K. Breeden. 2012b. Nitrogen-enhanced efficacy of mesotrione and topramezone for smooth crabgrass (*Digitaria ischaemum*) control. Weed Sci. 60:480–485.

Fuerst, E. P. and M. A. Norman. 1991. Interactions of herbicides with photosynthetic electron transport. Weed Sci. 39:458–464.
Grossmann, K. and T. Ehrhardt. 2007. On the mechanism and selectivity of the corn herbicide topramezone: a new inhibitor of 4-hydroxyphenylpyruvate dioxygenase. Pest Manag. Sci. 63:429–439.
Hugie, J. A., G. A. Bollero, P. J. Tranel, and D. E. Riechers. 2008. Defining the rate requirements for synergism between mesotrione and atrazine in redroot pigweed (*Armaranthus retroflexus*). Weed Sci. 56:265–270.
Johnson, B. J. 1983. Response to ethofumesate of annual bluegrass (*Poa annua*) and overseeded bermudagrass (*Cynodon dactylon*). Weed Sci. 31:385–390.
Joshi, P., S. Garita, M. K. Pradhan, and B. Biswal. 2011. Photosynthetic response of clusterbean chloroplasts to UV-B radiation: energy imbalance and loss in redox homeostasis between QA and QB of photosystem II. Plant Sci. 181:90–95.
Kaminski, J. E. and P. H. Dernoeden. 2007. Seasonal *Poa annua* L. seedling emergence patterns in Maryland. Crop Sci. 47:775–781.
Kim, J., S. Jung, I. T. Hwang, and K.Y. Cho. 1999. Characteristics of chlorophyll *a* fluorescence induction in cucumber cotyledons treated with diuron, norflurazon, and sulcotrione. Pestic. Biochem. Physiol. 65:73–81.
Lee, D. L., M. P. Prisbylla, T. H. Cromartie, D. P. Dagarin, S. W. Howard, W. M. Provan, M. K. Ellis, T. Fraser, and L. C. Mutter. 1997. The discovery and structural requirements of inhibitors of p-hydroxyphenylpyruvate dioxygenase. Weed Sci. 45:601–609.
McCullough, P. E., S. E. Hart, D. Weisenberger, and Z. J. Reicher. 2010. Amicarbazone efficacy on annual bluegrass and safety on cool-season turfgrasses. Weed Technol. 24:461–470.
McDonald, S. J., P. H. Dernoeden, and J. E. Kaminski. 2006. Creeping bentgrass tolerance and annual bluegrass control with bispyribac-sodium tank-mixed with iron and nitrogen. Appl. Turfgrass Sci. doi:10.1094/ATS-2006-0811-01-RS
McElroy, J. S., R. H. Walker, G. R. Wehtje, and E. van Santen. 2004. Annual bluegrass (*Poa annua*) populations exhibit variation in germination response to temperature, photoperiod, and fenarimol. Weed Sci. 52:47–52.
McIntosh, M. S. 1983. Analysis of combined experiments. Agron. J. 75:153–155.
Meyer, J. W. and B. E. Branham. 2006. Response of four turfgrass species to ethofumesate. Weed Technol. 20:123–129.
Mitchell, G., D. W. Bartlett, T.E.M. Fraser, T. R. Hawkes, D. C. Holt, J. K. Townson, and R. A. Wichert. 2001. Mesotrione: a new selective herbicide for use in maize. Pest Manag. Sci. 57:120–128.
Niyogi, K. K. 1999. Photoprotection revisited: genetic and molecular approaches. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:333–359.
Pallett, K. E. and A. J. Young. 1993. Carotenoids. Pages 59–90 *in* R. G. Alscher and J. J. Hess. eds. Antioxidants in Higher Plants. Boca Raton, FL: CRC Press.
Patton, A., D. Weisenberger, and B. Branham. 2011. Effect of Tenacity on annual bluegrass control in Kentucky bluegrass. West Lafayette, IN: 2010 Purdue University Research Summary. p. 19–20.
Perry, D. H., J. S. McElroy, and R. H. Walker. 2011. Effects of soil vs. foliar application of amicarbazone on annual bluegrass (*Poa annua*). Weed Technol. 25:604–608.
Pfündel, E. E., R. Pan, and R. A. Dilley. 1992. Inhibition of violaxanthin deepoxidation by ultraviolet-B radiation in isolated chloroplasts and intact leaves. Plant Physiol. 98:1372–1380.
Reicher, Z. J., D. V. Weisenberger, D. E. Morton, B. E. Branham, and W. Sharp. 2011. Fall applications of mesotrione for annual bluegrass control in Kentucky bluegrass. Appl. Turfgrass Sci. doi: 10.1094/ATS-2011-0325-01-RS
Senseman, S. A. 2007. Herbicide handbook. 9th ed. Lawrence, KS: Weed Science Society of America. 458 p.
Skelton, J. J., W. Sharp, and B. E. Branham. 2012. Postemergence control of annual bluegrass with mesotrione in Kentucky bluegrass. HortScience 47:522–526.
Strid, A., W. S. Chow, and S. M. Anderson. 1994. UV-B damage and protection at the molecular level in plants. Photosynth. Res. 39:475–489.
Sutton, P., C. Richards, L. Buren, and L. Glasgow. 2002. Activity of mesotrione on resistant weeds in maize. Pest Manag. Sci. 58:981–984.
Woodyard, A. J., J. A. Hugie, and D. E. Riechers. 2009. Interactions of mesotrione and atrazine in two weed species with different mechanisms for atrazine resistance. Weed Sci. 57:369–378.
Zhang, X., E. H. Ervin, and R. E. Schmidt. 2005. The role of leaf pigment and antioxidant levels in UV-B resistance of dark- and light-green Kentucky bluegrass cultivars. J. Am. Soc. Hortic. Sci. 130:836–841.

Received October 18, 2012, and approved April 30, 2013.