Annual Bluegrass (*Poa annua* L.) Control and Turfgrass Response to Amicarbazone

by

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Abstract

Amicarbazone is a photosystem II (PSII)-inhibiting herbicide being evaluated for annual bluegrass control in bermudagrass (*Cynodon* spp.) overseeded with perennial ryegrass (*Lolium perenne* L.). The objectives of this study were to: 1. evaluate the effects of soil versus foliar application of amicarbazone on annual bluegrass, 2. determine cool-season turfgrass response to amicarbazone as affected by temperature, 3. evaluate amicarbazone application timing on annual bluegrass control and perennial ryegrass response in overseeded bermudagrass, 4. evaluate triazine-resistant annual bluegrass populations for potential cross-resistance to amicarbazone, and 5. evaluate field applications of amicarbazone for annual bluegrass control and perennial ryegrass response in overseeded bermudagrass.

Annual bluegrass foliage and/or root exposure to amicarbazone reduced annual bluegrass quantum yield (Φ_{PSII}) causing plant death. However, soil exposure was more beneficial for annual bluegrass control. Data from temperature studies suggests as temperatures increased and amicarbazone rate increased, perennial ryegrass and annual bluegrass injury increased. Reduction in Φ_{PSII} of both species suggests PSII of both species was sensitive to amicarbazone regardless of temperature.

Amicarbazone application timing studies indicated applications 16 weeks after overseeding (WAO) result in the best combination of perennial ryegrass safety and annual bluegrass control. Amicarbazone/ethofumesate tank-mixes provided similar annual bluegrass control to sequential ethofumesate applications. Amicarbazone applied at 0.53 kg ha^{-1} in March resulted in the best combination of annual bluegrass control and perennial ryegrass safety; however, these applications were inferior to ethofumesate and sequentially-applied bispyribac.

Amicarbazone did not control triazine-resistant annual bluegrass populations in the cross resistance study. Quantum yield data of triazine-susceptible populations suggests amicarbazone efficiently inhibited PSII immediately following treatment. These data suggest triazine-resistant annual bluegrass populations may possess cross-resistance to amicarbazone.

This research indicates amicarbazone is a potent PSII inhibitor that potentially provides postemergence annual bluegrass control in bermudagrass overseeded with perennial ryegrass; however, currently registered herbicides may be more efficacious. These studies suggest amicarbazone applications in the spring result in greater annual bluegrass control with greater perennial ryegrass tolerance. Based on this research, factors such as herbicide placement, temperature, and application timing may affect amicarbazone efficacy.

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List of Abbreviations and Symbols

AB	Annual Bluegrass
ai	Active Ingredient
AL	Alabama
C	Celsius Degrees
DAA	Days After Application
DBO	Days Before Overseeding
DF	Dry Flowable
fb	Followed By
F'_m	Maximum Fluorescence in Lighted Conditions
F_t	Steady State Yield of Fluorescence in the Light
g	Gram
h	Hour
ha	Hectare
HAA	Hours After Application
kg	Kilogram
kPa	Kilopascals
L	Liter
LSD	Least Significant Difference
m	Meter

min	Minute
mo	Month
MOA	Mode of Action
MS	Mississippi
NC	North Carolina
NDVI	Normalized Difference Vegetative Index
N	Nitrogen
OR	Oregon
PBU	Plant Breeding Unit
POST	Postemergence
PR	Perennial Ryegrass
PRE	Preemergence
PSII	Photosystem II
s	Second
SC	Soluble Concentrate
TGRU	Turfgrass Research Unit
TN	Tennessee
USGA	United States Golf Association
v	Volume
VA	Virginia
WAO	Weeks After Overseeding
WAIT	Weeks After Initial Treatment
WAT	Weeks After Treatment

WBO	Weeks Before Overseeding
μmol	Micromole
Φ_{PSII}	Quantum Yield of photosystem II

I. Annual Bluegrass Control in Bermudagrass Overseeded and Not Overseeded With Perennial Ryegrass: A Review

Introduction

Annual bluegrass (*Poa annua* L.) is a prolific cool-season weed species which infests fine turfgrass. In most cases, annual bluegrass possesses a lighter-green color than the desired turfgrass species. Annual bluegrass produces numerous panicles which disrupt turfgrass aesthetics and supply the seed bank for future infestations (Beard 1973). Annual bluegrass control is complicated by the lack of a well-defined germination period. Germination in autumn and spring is well-documented in turf; however, the primary window of germination may be influenced by geographic region or a host of environmental factors (Branham 1991; Callahan and McDonald 1992; Dernoeden 1998; Vargas and Turgeon 2004). Seasonal emergence studies in Maryland reported 50-70% annual bluegrass emergence between September and October, and 24% emergence between November and May (Kaminski and Dernoeden 2007).

Annual bluegrass chemical control was reported as early as the 1930’s, and continues to be researched extensively (Sprague and Burton 1937; McElroy et al. 2011). Numerous herbicides are available that control annual bluegrass; however, acceptable control without injuring turfgrass continues to be a challenge in certain turfgrass species (McElroy et al. 2011; McCullough et al. 2010). Numerous herbicides have been utilized for annual bluegrass control in various turfgrass scenarios; therefore, this paper will concentrate on control in dormant, non-overseeded, warm-season turf and warm-season turf overseeded with perennial ryegrass.

Non-Overseeded Bermudagrass

Postemergence (POST) Annual Bluegrass Control in Dormant Bermudagrass

Annual bluegrass control can be achieved in completely dormant bermudagrass with little concern of turfgrass injury. Non-selective herbicides such as glyphosate, glufosinate, and paraquat provide acceptable annual bluegrass control and minimal detrimental effects on spring bermudagrass transition when applied during complete bermudagrass dormancy (Harrell et al. 2008; Toler et al. 2007; Walker et al. 2003; Johnson 1976a). Glyphosate controlled annual bluegrass greater with February applications than December applications (Toler et al. 2007). Application of these non-selective herbicides during periods of quasi-dormancy could result in delayed bermudagrass green-up the following spring (McCarty 2005).

Sulfonylurea herbicides are ALS (acetolactate synthase)-inhibiting herbicides which selectively control some grass species in turfgrass (Larocque and Christians 1985). Rimsulfuron was registered for annual bluegrass control in bermudagrass turf in 2001 and hybrid bermudagrass putting greens in 2002 (Walker et al. 2003). Rimsulfuron controlled mature annual bluegrass greater as rates increased from 0.016 to 0.048 kg ha^{-1} (Walker et al. 2003). Preemergence (PRE) activity has also been observed with rimsulfuron (Walker et al. 2003; Wehtje and Walker 2002). Persistence of rimsulfuron on treated turf is responsible for lateral movement of rimsulfuron from treated areas (Barker et al. 2006). Trifloxysulfuron, foramsulfuron, and flazasulfuron applied at 0.03, 0.028, and 0.06 kg ha^{-}

^{1} , respectively, controlled annual bluegrass >90% in dormant bermudagrass (Harrell et al. 2008; Toler et al. 2007). Foramsulfuron applied in December controlled annual bluegrass 60% (Yelverton 2003).

PRE Annual Bluegrass Control in Bermudagrass

Oxadiazon applied at 3.4 to 4.5 kg ha^{-1} provided > 88% control for three straight years when applied PRE (Johnson 1976b). Oxadiazon applied at 3.4 kg ha^{-1} in August resulted in >90% control of annual bluegrass (Bingham and Shaver 1979). Oxadiazon applied twice at 4.5 kg ha^{-1} retarded bermudagrass putting green growth the following spring, while single oxadiazon applications at 4.5 and 6.7 kg ha^{-1} did not affect spring bermudagrass growth with >90% annual bluegrass control (Johnson 1983a; Johnson 1977).

Indaziflam is a cell wall biosynthesis inhibitor which provides early POST and PRE control of annual bluegrass. Indaziflam applied at 40 to 60 g ha^{-1} provided near complete control of annual bluegrass 24 to 28 WAT (Perry et al. 2011; Cooper et al. 2010; Myers et al. 2009). Applications in late fall and spring were less efficacious in controlling annual bluegrass (Perry et al. 2011; Jester et al. 2009).

Pronamide exhibits both POST and PRE activity and should be applied in fall or early winter at 0.6-1.7 kg ha^{-1} (Anonymous 2009). Pronamide applied at 0.8 kg ha^{-1} in October resulted in >93% annual bluegrass control the following spring for two straight years (Johnson 1975; Johnson 1976b; Johnson 1977). February pronamide applications at 1.68 kg ha^{-1} controlled annual bluegrass 99% (Toler et al. 2007).

Simazine and atrazine are both s-triazine herbicides that provide economical POST and PRE control of annual bluegrass when applied at 1.1 to 2.2 kg ha^{-1}. Simazine at the lowest label rates has achieved up to 95% annual bluegrass control (Hutto et al. 2004). Simazine applied at 0.9 to 2.2 kg ha^{-1} in September and February completely controlled annual bluegrass (Johnson 1982).

Other PRE herbicides that are widely utilized and provide good annual bluegrass control in dormant, non-overseeded turf include: pendamethalin, prodiamine, dithiopyr, and oryzalin. Pendamethalin, prodiamine, and oryzalin are dinitroanaline herbicides which inhibit microtubulin production in susceptible species (Senseman 2007). Johnson and Murphy (1995) reported near complete control of annual bluegrass with the aforementioned PRE herbicides. For the purposes of this paper, several of these herbicides will be discussed further in the following section.

Bermudagrass Overseeded with Perennial Ryegrass

Overseeding practices include seeding perennial ryegrass into actively growing bermudagrass in late summer to early fall. The primary goal is to achieve a seamless transition from an actively growing warm-season turf to an actively growing cool-season turf. Chemical control of annual bluegrass in overseeding situations is complicated by the fact that annual bluegrass begins to germinate near traditional overseeding dates. Herbicides utilized in these situations must provide annual bluegrass control and perennial ryegrass safety. McElroy et al. (2011) characterized three schemes of annual bluegrass control in perennial ryegrass overseed: (1) PRE herbicides applied before overseeding, (2) sulfonylurea herbicides applied before overseeding, and (3) POST selective herbicides.

POST Herbicides Applied After Overseeding

Postemergence annual bluegrass control in overseeded bermudagrass requires grass in grass selectivity; a characteristic which many herbicides do not possess in this particular scenario. Currently, ethofumesate and bispyribac-sodium (bispyribac) are the only herbicides registered for POST annual bluegrass control in bermudagrass overseeded with perennial ryegrass.

Ethofumesate has been utilized since the early 1980’s for POST annual bluegrass control in overseeding situations. Complete bermudagrass dormancy is required when making ethofumesate applications, or immediate injury may result and spring bermudagrass transition may be delayed (Johnson 1983b; McCarty 2008). Dickens (1979) observed bermudagrass injury following fall applications, but did not observe bermudagrass transition issues the following spring. Rossi (2001) reported annual bluegrass control, but unacceptable perennial ryegrass injury when applied three times at two week intervals. Yelverton and McCarty (2001) observed > 95% annual bluegrass control following ethofumesate applications 6 and 9 WAO. McElroy et al. (2011) achieved > 90% annual bluegrass control with ethofumesate applied sequentially 12 and 15 WAO. Ethofumesate success may be attributed to annual bluegrass’ ability to absorb more herbicide than perennial ryegrass (Kohler and Branham 2002).

Bispyribac is registered for POST annual bluegrass control in bermudagrass overseeded with perennial ryegrass (Anonymous 2008). Temperature is reported to affect bispyribac activity on annual bluegrass (McCullough and Hart 2006). McElroy et al. (2011) observed acceptable annual bluegrass control with single and sequential bispyribac applications, but sequential applications significantly reduced perennial ryegrass cover when applied 12 and 15 WAO. Amicarbazone is a photosystem II (PSII) inhibiting herbicide being evaluated for annual bluegrass control in bermudagrass overseeded with perennial ryegrass (Figure 1). Walker and Belcher (2009) reported near complete control of annual bluegrass with March applications. Control was achieved with single or sequential amicarbazone applications at 0.52 and 0.26 kg ha^{-1}, respectively, with no adverse effects on spring bermudagrass transition (Walker and Belcher 2009).

POST Herbicides Applied Before Overseeding

Foramsulfuron is labeled for application 1 week before overseeding (WBO) (Anonymous 2007). Foramsulfuron applied at 17.5 to 35 g ha^{-1} 2 WBO provided 80-90% annual bluegrass control 20 WAA with minimal perennial ryegrass injury (Brecke et al. 2005). McElroy et al.(2011) reported 63% panicle reduction with inadequate annual bluegrass control following foramsulfuron applications 2 WBO. Other herbicides such as rimsulfuron, sulfosulfuron, and trifloxysulfuron reportedly control annual bluegrass initially, but additional herbicide applications may need to be applied 20 WAIA (McCarty 2008; Brecke et al. 2005).

PRE Herbicides Applied Before Overseeding

Prodiamine applied at 0.56-1.1 kg ha^{-1} at 6 to 8 WBO provided good annual bluegrass control and perennial ryegrass safety regardless of rate (Yelverton and McCarty 1999). Prodiamine applied at 0.56 and 0.84 kg ha^{-1} 60 days before overseeding (DBO) controlled annual bluegrass >90% the following spring (McCarty 2008). In the same study, prodiamine applied at 0.43 followed by (fb) 0.43 kg ha^{-1} 60 DBO and January controlled annual bluegrass 93%.

Pendamethalin and benefin applied at 2.2 and 3.1 kg ha^{-1} 8 WBO, respectively, provided 74 and 84% annual bluegrass control with minimal perennial ryegrass injury (Yelverton and McCarty 2001). Dithiopyr applied at 0.28 or 0.56 kg ha^{-1} 4 or 8 WBO was safe on perennial ryegrass (Yelverton and McCarty 2001). Oxadiazon applied at 2.2 kg ha^{-1} 8 WBO was safe on perennial ryegrass, but 4.4 kg ha^{-1} reduced perennial ryegrass establishment (Yelverton and McCarty 2001).

Research Justification

Annual bluegrass commonly plagues bermudagrass overseeded with perennial ryegrass, and few POST control options are available to turfgrass practitioners. Currently, two herbicides are registered for annual bluegrass control in bermudagrass overseeded with perennial ryegrass: bispyribac and ethofumesate. These herbicides control annual bluegrass in this scenario, but some factors may affect their efficacy and safety in desirable turfgrass. Ethofumesate should be applied during complete bermudagrass dormancy or unacceptable bermudagrass injury or spring transition may result. As a result, ethofumesate use in many areas is confined to December and January applications.

Bispyribac labeling recommends confining applications to when temperatures are below 13 C and not expected to exceed 13 C within 3 DAA. Amicarbazone is a herbicide being investigated for use in bermudagrass overseeded with perennial ryegrass. Preliminary research indicates amicarbazone possesses selective POST activity on annual bluegrass in bermudagrass overseeded with perennial ryegrass with minimal bermudagrass injury (Walker and Belcher 2009).

Objectives

The objectives of this study were to: 1. Determine amicarbazone effects on annual bluegrass and creeping bentgrass response in a putting green scenario, 2. Evaluate the effects of soil versus foliar application of amicarbazone on annual bluegrass, 3. Determine cool-season turfgrass response to amicarbazone as affected by temperature, 4. Evaluate amicarbazone application timing affects on annual bluegrass control and perennial ryegrass response in bermudagrass overseeded with perennial ryegrass, 5. Evaluate triazine-resistant annual bluegrass populations for potential cross-resistance with amicarbazone, and 6. Evaluate field applications of amicarbazone for annual bluegrass control and perennial ryegrass response in bermudagrass overseeded with perennial ryegrass.

Literature Cited

Anonymous. 2007. Revolver^{TM} Herbicide Label. Bayer Environmental Science. 2 T. W. Alexander DR., RTP, NC 27709.

Anonymous. 2008. Velocity® SG Herbicide Label. Valent U.S.A. Corporation. Walnut Creek, CA 94596.

Anonymous. 2009. Kerb® 50WP Herbicide Label. Dow AgroSciences LLC. 9330 Zionsville Road, Indianapolis, IN 46268.

Barker, W. L., J. B. Beam, and S. D. Askew. 2006. Persistence of rimsulfuron on perennial ryegrass (*Lolium perenne*) and annual bluegrass (*Poa annua*) foliage. Weed Technol. 20:345-350.

Beard, J. B. 1973. Turfgrass: Science and Culture. Englewood Cliffs, NJ, Prentice-Hall. Pp. 68-69.

Bingham, S. W. and R. L. Shaver. 1979. Effectiveness of herbicide programs for annual bluegrass (*Poa annua*) control in bermudagrass (*Cynodon dactylon*). Weed Sci. 27:367-371.

Branham, B. 1991. Dealing with *Poa annua*. Golf Course Manage. 59:46-60.

Brecke, B., D. O. Stephenson, and J. B. Unruh. 2005. Annual bluegrass control in overseeded bermudagrass. ASA-CSSA-SSSA International Meeting. No. 64-9.

Callahan, L. M., and E. R. McDonald. 1992. Effectiveness of bensulide in controlling two annual bluegrass (*Poa annua*) subspecies. Weed Technol. 6:97-103.

Cooper, T., A. Hephner, T. Williams, L. Beck, and G. Henry. 2010. Pre- and postemergence annual bluegrass control with indaziflam. CSSA Abstract.

Dernoeden, P. H. 1998. Use of prodiamine as a preemergence herbicide to control annual bluegrass in Kentucky bluegrass. HortSci. 3:845-846.

Dickens, R. 1979. Control of annual bluegrass (*Poa annua*) in overseeded bermudagrass (*Cynodon* spp.) golf greens. Weed Sci. 27:642-644.

Harrell, III, J. R., A. G. Estes, and L. B. McCarty. 2008. Annaul bluegrass (*Poa annua*) control in overseeded and non-overseeded bermudagrass turf. Proc. South. Weed Sci. Soc. 61:113.

Hutto, K. C., G. E. Coats, and J. M. Taylor. 2004. Annual bluegrass (*Poa annua*) resistance in Mississippi. Weed Technol. 18:846-849.

Jester, J. L., J. B. Willis, M. J. Goddard, T. L. Mittlesteadt, and S. D. Askew. 2009. Annual bluegrass control with AE 717. Proc. Weed Sci. Soc. Amer.

Johnson, B. J. 1975. Dates of herbicide application for weed control in bermudagrass. Weed Sci. 23:110-115.

Johnson, B. J. 1976a. Glyphosate for weed control in dormant bermudagrass. Weed Sci. 24:140-143.

Johnson, B. J. 1976b. Herbicides for seasonal weed control in turfgrasses. Agron. J.. 68:717-720.

Johnson, B. J. 1977. Preemergence winter weed control in dormant bermudagrass turf. Agron. J.. 69:573-576.

Johnson, B. J. 1982. Simazine formulation treatments on control of winter weeds in bermudagrass turf. Agron. J. 74:881-886.

Johnson, B. J. 1983a. Tolerance of bermudagrass (*Cynodon dactylon*) putting greens to herbicide treatments. Weed Sci. 31:415-418.

Johnson, B. J. 1983b. Response to ethofumesate of annual bluegrass (*Poa annua*) and overseeded bermudagrass (*Cynodon dactylon*). Weed Sci. 31:385-390.

Johnson, B. J. and T. R. Murphy. 1995. Winter annual weed control with herbicides in turfgrasses. Bulletin 421. [Online] http://commodities.caes.uga.edu/turfgrass/georgiaturf/WeedMngt/weedcontrol/bull421.htm

Kaminski, J. E. and P. H. Dernoeden. 2007. Seasonal *Poa annua* L. seedling emergence patterns in Maryland. Crop Sci. 47:775-781.

Kohler, E. A. and B. E. Branham. 2002. Site of uptake, absorption, translocation, and metabolism of ethofumesate in three turfgrass species. Weed Sci. 50:576-580.

Larocque, D. J. and N. E. Christians. 1985. Selective control of tall fescue in Kentucky bluegrass with chlorsulfuron. Agron. J.. 77:86-89.

McCarty, L. B. 2008. For successful control, know your *Poa annua*. Golf Course Manage. 76:111-115.

McCarty, L. B. 2005. Best Golf Course Management Practices 2nd ed. Pearson Prentice Hall. Upper Saddle River, NJ.

McCullough, P. E., and S. E. Hart. 2006. Temperature influences creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*) response to bispyribac-sodium. Weed Technol. 20:728-732.

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.

McElroy, J. S., G. K. Breeden, and G. Wehtje. 2011. Evaluation of annual bluegrass control programs for bermudagrass turf overseeded with perennial ryegrass. Weed Technol. 25:58-63.

Myers, D. F., R. Hanrahan, J. Michel, B. Monke, L. Mudge, L.Norton, C. Olsen, A. Parker, J. Smith, and D. Spak. 2009. Indaziflam/BCS-AA10717-A new herbicide for pre-emergent control of grasses and broadleaf weeds for turf and ornamentals. Proc. South. Weed Sci. Soc. 62:393.

Perry, D. H., J. S. McElroy, M. C. Doroh, and R. H. Walker. 2011. Indaziflam utilization for controlling problematic turfgrass weeds. Applied Turfgrass Science. (In Press).

Rossi, F. S. 2001. Annual bluegrass population dynamics in response to growth regulators and herbicides. Int. Turfgrass Soc. Res. J. 9:906-909.

Senseman, S.A. 2007. Herbicide Handbook. 9th ed. Lawrence, KS. Weed Science Society of America. Pp. 265-286.

Sprague, H. B. and G. W. Burton. 1937. Annual bluegrass (*Poa annua* L.) and its requirements for growth. NJ Agric. Exp. Sta. Bull. 630:24.

Toler, J. E., T. G. Willis, A. G. Estes, and L. B. McCarty. 2007. Postemergent annual bluegrass control in dormant non-overseeded bermudagrass turf. HortSci. 42:670-672.

Vargas, J. M., Jr., and A. J. Turgeon. 2004. *Poa annua*: Physiology, culture, and control of annual bluegrass. John Wiley & Sons, Hoboken, NJ. Pp. 22-23.

Walker, R. H. and J. L. Belcher. 2009. Annual bluegrass control with amicarbazone in perennial ryegrass overseed. Proc. of South. Weed Sci. Soc. 62:191.

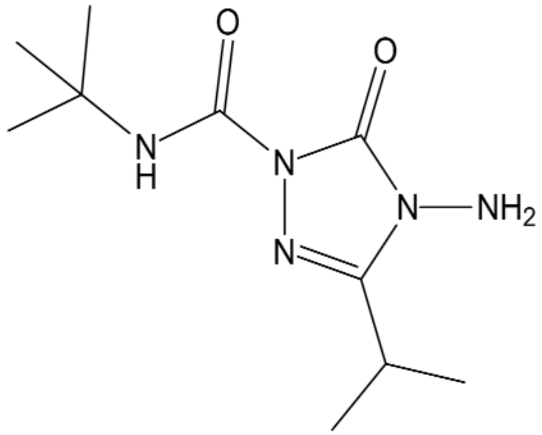
Walker, R. H., G. R. Wehtje, and J. L. Belcher. 2003. *Poa annua* control with rimsulfuron. Golf Course Management.71:120-123.

Wehtje, G. and R. H. Walker. 2002. Response of two annual bluegrass varieties to preemergence and postemergence rimsulfuron. Weed Technol. 16:612-616.

Yelverton, F. H. 2003. A new herbicide for weeds in bermudagrass and zoysiagrass. Golf Course Manage. 71:119-122.

Yelverton, F. H. and L. B. McCarty. 2001. Tolerance of perennial ryegrass and *Poa annua* control with herbicides in overseeded bermudagrass. Int. Turfgrass Soc. Res. J. 9:1050-1055.

Figure 1. Chemical structure of amicarbazone.



II. Annual Bluegrass and Creeping Bentgrass Photosynthetic Response to Amicarbazone in Putting Green Conditions

Abstract

Amicarbazone is a photosystem II inhibiting herbicide which is being investigated for annual bluegrass control in creeping bentgrass putting greens. However, recent studies suggest amicarbazone efficacy may vary between seasons and temperatures. Photosynthetic effects of amicarbazone to these grass species may provide insight to further develop annual bluegrass control programs. Therefore, experiments were conducted between fall 2007 and spring 2009 in Tennessee and AL to investigate annual bluegrass and creeping bentgrass photosynthetic response to amicarbazone applications. Herbicide treatments included single applications of amicarbazone at 0.1, 0.2, 0.3, or 0.4 kg ai ha^{-1} and paclobutrazol at 0.14 or 0.28 kg ha^{-1} were included as standard treatments. Annual bluegrass quantum yield (Φ_{PSII}) was reduced with all amicarbazone applications, but this reduction increased with increasing herbicide rate and spring applications. Creeping bentgrass Φ_{PSII} was reduced greatest in the fall studies, while creeping bentgrass recovery was observed in the spring 2 WAT. Creeping bentgrass injury and reduction in cover was greatest following fall applications. Results of these studies suggest Φ_{PSII} of annual bluegrass and creeping bentgrass is reduced with

amicarbazone and photosystem II recovery may depend on the season of application or environmental conditions during those seasons.

Introduction

Creeping bentgrass is a fine-textured turfgrass species commonly utilized on golf course putting greens from the cool-humid to warm-humid climatic regions. Frequent irrigation, fertilization, and fungicide applications on these greens enhance the survivability of annual bluegrass (Beard 1973). Mixed stands of annual bluegrass and creeping bentgrass are aesthetically unappealing due to differences in green color and overall reduction in uniformity. Annual bluegrass produces copious seedheads when conditions are favorable and seed are incorporated through the turf canopy to promote future germination and infestation.

Certain herbicides successfully control annual bluegrass on creeping bentgrass greens; however, resulting injury may be considered unacceptable. Bispyribac-sodium (bispyribac) provides acceptable annual bluegrass control, but unacceptable creeping bentgrass injury may result at low mowing heights (Teuton et al. 2007; McCullough and Hart 2009). McCullough and Hart (2006) reported decreased creeping bentgrass injury at cooler temperatures with bispyribac but reduced annual bluegrass control. Bispyribac applications to fairway-height creeping bentgrass induce chlorosis; however, this injury can be alleviated with iron and nitrogen applications (McDonald et al. 2006). Some PRE herbicides labeled for control of annual grasses do not successfully control perennial, annual bluegrass biotypes (*Poa annua* spp. *reptans*) (Dernoeden 2000). Plant growth regulators such as paclobutrazol are used to suppress annual bluegrass growth while inflicting minimal creeping bentgrass injury (Johnson and Murphy 1995; Isgrigg et al. 1998).

Amicarbazone [4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] is a triazolinone herbicide which controls susceptible weeds by inhibiting photosystem II (PSII) (Dayan et al. 2009). Grasses and broadleaves are included in its spectrum of weed control. Amicarbazone does have residual activity which broadens its uses in PRE and POST situations (Senseman 2007). To date, it has been utilized in PRE and POST applications in sugar cane (*Saccharum officinarum* L.) and preplant or PRE in corn (*Zea mays* L.). Velvetleaf (*Abutilon theophrasti* Medik), common lambsquarters (*Chenopodium album* L.), and marmaladegrass [*Brachiaria plantaginea* (Link) Hitch.] are a few species controlled with amicarbazone (Philbrook et al. 1999; Senseman 2007). Symptoms of affected plants are similar to other PSII-inhibiting herbicides and include chlorosis, stunted growth, tissue necrosis, and eventual death (Senseman 2007; Dayan et al. 2009). Amicarbazone applied sequentially at 131 g ha^{-1} in April was reported to provide good annual bluegrass control; however, higher rates and sequential applications in the fall resulted in excessive creeping bentgrass injury and stand reduction (Warren et al. 2009).

Rate and time of year are reported to have an influence on creeping bentgrass phytotoxicity to amicarbazone, and was exacerbated by sequential applications (McCullough et al. 2010). Creeping bentgrass and annual bluegrass are both susceptible to greater injury as temperatures increase from 10 to 30 C (McCullough et al. 2010). Research is warranted to investigate the photosynthetic response of creeping bentgrass and annual bluegrass following treatment with amicarbazone in order to establish amicarbazone application regimes. Objectives of these field experiments were 1) to investigate the physiological and phenotypic response of creeping bentgrass following single amicarbazone applications in spring and fall, and 2) to investigate annual bluegrass physiological response following single amicarbazone applications in spring and fall.

Materials and Methods

Five field experiments (Table 1) were conducted at separate locations between fall 2007 and spring 2009. Two studies were conducted in fall 2007: one study was conducted at the East Tennessee Research and Education Center in Knoxville, Tennessee on a research green consisting of a monoculture of 'Crenshaw 'creeping bentgrass mown at approximately 3.3 mm. The other fall 2007 study was conducted at Knoxville Municipal Golf Course on a mixed stand of 'Crenshaw' creeping bentgrass and a perennial biotype of annual bluegrass (*Poa annua* spp. *reptans*) mown at approximately 3.2 mm. The root zone was a modified-push-up green which had been topdressed regularly to incorporate sand into the root zone. The spring 2008 study was conducted at the Auburn University Turfgrass Research Unit in Auburn, AL on a research green consisting of a mixed stand of 'G2' creeping bentgrass and *P. annua* spp. *reptans*. The soil was a Marvyn sandy loam (Marvyn fine-loamy, kaolinitic, thermic Typic Kanhapludults) with 6.1 pH. Research areas were fertilized with 19 kg N ha^{-1} mo^{-1} and mown daily at 3.2 mm. Fall 2008 and spring 2009 studies were conducted at Grand National Golf Course in Opelika, AL on a 2-year old putting green with a pure stand of 'Dominant' creeping bentgrass. The soil profile was 80% USGA sand and 20% Profile[1].

Turf was fertilized with 20 kg N ha^{-1} mo^{-1} and mown daily at 3.3 mm. All research and putting greens were irrigated to maintain adequate moisture.

Herbicide treatments included single applications of amicarbazone[2] at 0.1, 0.2, 0.3, or 0.4 kg ha^{-1} and paclobutrazol[3] at 0.14 or 0.28 kg ha^{-1} were included as standard treatments. A non-treated check was included in all studies. Treatments were applied with 0.25% v/v nonionic surfactant[4] to 1 by 1 m plots with a CO_2 pressurized backpack sprayer using 8002VS nozzles[5] calibrated to deliver 280 L ha^{-1} of spray solution at 138 kPa. Herbicide applications were made on October 11, April 2, October 31, and March 18 for the fall 2007, spring 2008, fall 2008, and spring 2009 studies, respectively. All treatments were applied in the afternoon when dew was not present.

Quantum Yield (Φ_{PSII}) of Annual Bluegrass and Creeping Bentgrass. Quantum yield of annual bluegrass and creeping bentgrass was determined by measurements of treated plots utilizing a pulse-modulated chlorophyll fluorometer[6]. Φ_{PSII} is a useful parameter to utilize in the evaluation of plants that may be stressed due to a variety of biotic or abiotic factors (Krause and Weis 1991; Maxwell and Johnson 2000; Tyystjarvi et al. 1999). Φ_{PSII} is calculated by the formula:

$$\Phi_{PSII} = (F'_m - F_t)/F'_m$$

where F'_m equals maximum fluorescence in lighted conditions and F_t equals steady state yield of fluorescence in the light. Φ_{PSII} is a commonly used measurement for determining the proportion of light absorbed by chlorophyll associated with PSII that is utilized in photochemistry (Genty et al. 1989; Maxwell and Johnson 2000). It is considered by some researchers to be the best parameter to measure efficiency of PSII (Genty et al. 1989). Three Φ_{PSII} measurements were recorded for each plot by holding the light probe at approximately 45° directly above the turf canopy. Saturation pulse width and modulation intensity were set to 0.8 s and 4, respectively. Measurements taken during cloudy conditions were avoided to reduce variability between measurements. Φ_{PSII} was standardized relative to the non-treated plots using the formula:

$$\text{relative } \Phi_{PSII} = [(Y_{NT} - Y_T)/\ Y_{NT}] \times 100$$

where Y_T equaled Φ_{PSII} of treated plants, and Y_{NT} equaled Φ_{PSII} of non-treated plants. Annual bluegrass in mixed sward sites (Knoxville Municipal Golf Course and Auburn University Turfgrass Research Unit) was the only species to undergo Φ_{PSII} measurements, and creeping bentgrass Φ_{PSII} was measured on all study sites with a creeping bentgrass monoculture. Φ_{PSII} data were recorded weekly through 6 WAT.

Visual Ratings and Digital Image Analysis. All experimental plots were visually evaluated for creeping bentgrass injury on a percentage scale, where 0 equaled no chlorosis or necrosis and 100 equaled complete death of creeping bentgrass (Frans et al. 1986). Digital image analysis was utilized for calculating percent creeping bentgrass cover. A portable light box (61 cm long, 51 cm wide, and 56 cm high) was used in association with a digital camera to obtain 1 megapixel digital images of each plot. Images were analyzed using Sigma Scan Pro software[7] to determine percent creeping bentgrass cover, where 100 equaled complete cover and 0 equaled no creeping bentgrass (Karcher and Richardson 2005). Creeping bentgrass injury and cover data were collected 2, 4, and 6 WAT on pure creeping bentgrass greens only.

Experiments were organized in randomized complete block designs with four replications. Data for each study were tested for normality using residual plots in PROC GLIMMIX of SAS[8] and normality was satisfied. Least square means of Φ_{PSII} data were evaluated in PROC MIXED. Significance of main effects for injury and cover data were analyzed at $\alpha = 0.05$ in PROC GLM and means were separated using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Quantum Yield (Φ_{PSII}) **of Annual Bluegrass and Creeping Bentgrass.** Annual bluegrass Φ_{PSII} in the fall 2007 and spring 2008 studies were significantly different ($P < 0.001$); therefore, data for the two studies will be presented separately. A significant trial by treatment interaction ($P < 0.001$) existed for fall 2007 and spring 2008 studies. In fall 2007, amicarbazone applied at 0.30 and 0.40 kg ha^{-1} reduced Φ_{PSII} of annual bluegrass 53 and 56% relative to the non-treated, respectively 2 WAT (Table 2). Amicarbazone applied at 0.10 kg ha^{-1} reduced annual bluegrass Φ_{PSII} 26% 2 WAT. All amicarbazone treated annual bluegrass began recovery 2 WAT. Annual bluegrass treated with amicarbazone at 0.30 and 0.40 kg ha^{-1} recovered > 20% 4 WAT. Annual bluegrass treated with all rates of amicarbazone recovered to ≥ 88% of the non-treated 6 WAT. In spring 2008, amicarbazone applied at 0.40 kg ha^{-1} reduced annual bluegrass Φ_{PSII} 74% which was approximately 20% greater than the fall 2007 study (Table 2). Annual bluegrass Φ_{PSII} was reduced greater as amicarbazone rates increased from 0.10 to 0.40 kg ha^{-1}. Amicarbazone applied at 0.1 and 0.40 kg ha^{-1} reduced annual bluegrass Φ_{PSII} 31 and 74%, respectively 2 WAT. Annual bluegrass recovered ≥ 15% following all amicarbazone treatments 4 WAT. Amicarbazone applied at 0.2, 0.3, and 0.4 kg ha^{-1} reduced annual bluegrass Φ_{PSII} 40, 56, and 63%, respectively 6 WAT. Prolonged reduction in annual bluegrass Φ_{PSII} in the spring 2008 study may have been attributed to increasing temperatures observed during the spring study (Figure 2). However, <12% reduction in annual bluegrass quantum yield was observed in the fall 6 WAT. This is consistent with recent observations by McCullough et al. (2010), who observed an increase in annual bluegrass injury as temperatures increased from 10 to 30 C during growth chamber studies. They determined amicarbazone applied at 0.35 kg ha^{-1} would be needed to injure annual bluegrass 50% at 20 C and 0.06 kg ha^{-1} would be needed for 50% injury at 30 C. Temperatures at the time of application in our spring 2008 study were approximately 23 C and the research green experienced >24 C for 22 days during the study period. This may be partially responsible for differences in annual bluegrass recovery observed in the fall 2007 and spring 2008 studies (Figure 3). Paclobutrazol applied at 0.14 and 0.28 kg ha^{-1} did not significantly reduce annual bluegrass Φ_{PSII} for the fall 2007 or spring 2008 study. No visible annual bluegrass control was observed from single amicarbazone applications in either study.

Creeping bentgrass Φ_{PSII} was evaluated in fall 2007, fall 2008, and spring 2009. A significant treatment by season interaction ($P < 0.001$) was detected for creeping bentgrass Φ_{PSII} data; therefore, the fall studies were pooled (Table 3). Amicarbazone applied in the fall reduced creeping bentgrass Φ_{PSII} >20% for all rates 2 WAT. Amicarbazone applied at 0.4 kg ha^{-1} reduced creeping bentgrass Φ_{PSII} 54% 2 WAT. Amicarbazone applied at 0.1 to 0.4 kg ha^{-1} reduced creeping bentgrass Φ_{PSII} 13 to 33% 4 WAT, indicating some creeping bentgrass recovery. By 6 WAT, creeping bentgrass Φ_{PSII} had recovered to within 13% of non-treated levels. Although the fall 2007 studies and the fall 2008 study were in separate locations, Φ_{PSII} trends observed between annual bluegrass and creeping bentgrass were very similar (Figures 2 and 3). This suggests similar photosystem II responses between the two species. McCullough et al. (2010) observed similar responses among annual bluegrass and creeping bentgrass plants when grown under controlled growth chamber conditions. In spring 2009, creeping bentgrass Φ_{PSII} was reduced significantly with increasing amicarbazone rates 1 WAT (Figure 4). However, Φ_{PSII} levels recovered rapidly 2 WAT with increased Φ_{PSII} being observed with amicarbazone applied at 0.2 to 0.4 kg ha^{-1}. Creeping bentgrass Φ_{PSII} levels resulting from amicarbazone applications were reduced < 10% for the remainder of the evaluation period. Paclobutrazol applied at 0.14 or 0.28 kg ha^{-1} reduced creeping bentgrass Φ_{PSII} <8% throughout the evaluation period.

Seasonal differences in creeping bentgrass physiological response to amicarbazone may be attributed to the physiological status of the turf at the time of application. In early fall, bentgrass in the South is usually recovering from stressful, growing conditions from summer months and may be in a weakened state (Dernoeden 2000). Heat stress in summer months has the potential to reduce creeping bentgrass root health and render the turf more susceptible to herbicide injury in early fall. However, in spring bentgrass has undergone a recovery period between fall and spring, and the ability of creeping bentgrass to outgrow the deleterious effects of amicarbazone during this period may be possible.

Visual Ratings and Digital Image Analysis. A treatment by season of application interaction ($P < 0.001$) was detected for creeping bentgrass injury. As a result, injury data for the fall studies were pooled (Table 4). Creeping bentgrass injury following fall amicarbazone applications ranged from 4 to 44% with 0.1 to 0.4 kg ha^{-1}, respectively 2 WAT. By 4 WAT, injury from amicarbazone applied at 0.3 and 0.4 kg ha^{-1} was reduced from 33 to 18% and 44 to 33%, respectively. Amicarbazone applied at 0.1 and 0.2 kg ha^{-1} injured creeping bentgrass less than 11% throughout spring and fall studies. In the spring, amicarbazone applied at 0.1, 0.2, 0.3, and 0.4 kg ha^{-1} injured creeping bentgrass 5, 10, 20, and 21% respectively, 2 WAT. Creeping bentgrass recovery was observed through 6 WAT at which time injury was <10% for all amicarbazone treatments. Paclobutrazol applied at 0.28 kg ha^{-1} caused purple discoloration of the creeping bentgrass canopy resulting in 11% injury 6 WAT.

A significant treatment by season of application interaction was observed for creeping bentgrass cover data 2 WAT. Amicarbazone applied at 0.3 and 0.4 kg ha^{-1} reduced creeping bentgrass cover 21 and 32%, respectively 2 WAT while the same applications in the spring reduced cover 16 and 21% at the same rating interval (Table 5). Creeping bentgrass cover following amicarbazone applications was similar for spring and fall treatments 4 and 6 WAT. Amicarbazone applied at 0.4 kg ha^{-1} reduced creeping bentgrass cover 18 and 12% 4 WAT for the fall and spring studies, respectively. Amicarbazone applications reduced creeping bentgrass cover ≤10% 6 WAT for fall and spring studies. Amicarbazone applied at 0.1 or 0.2 kg ha^{-1} reduced creeping bentgrass cover <10% for fall and spring studies. Paclobutrazol applied at 0.14 and 0.28 kg ha^{-1} decreased creeping bentgrass cover 8 and 10%, respectively, 6 WAT in the fall studies.

Annual bluegrass control in creeping bentgrass putting greens continues to be a challenge for turfgrass managers. This can be partially attributed to a lack of herbicides that provide efficient annual bluegrass control in conjunction with good creeping bentgrass tolerance. Seasonal differences in herbicide activity observed in this study are not uncommon. Lycan and Hart (2006) observed excessive injury and reductions in quality of creeping bentgrass with spring and summer applications of bispyribac, but summer applications successfully reduced annual bluegrass populations while maintaining creeping bentgrass safety. McCullough et al. (2010) observed greater annual bluegrass control with fall applications of amicarbazone, but less creeping bentgrass injury with spring applications. However, research suggests that amicarbazone has the potential to be a viable option for golf course superintendents (McCullough et al. 2010; Yelverton 2008). The greatest level of creeping bentgrass safety was with amicarbazone applied at 0.1 and 0.2 kg ha^{-1}. Our studies suggest amicarbazone efficiently inhibits photosystem II of annual bluegrass and creeping bentgrass as indicated by the Φ_{PSII} data collected in these studies. Although PSII of creeping bentgrass was significantly inhibited in the fall and spring studies, creeping bentgrass was able to recover quicker following spring applications. Future investigations include utilizing Φ_{PSII} data to determine amicarbazone re-application intervals for establishing annual bluegrass control regimes in conjunction with optimizing creeping bentgrass safety.

Sources of Materials

[1] Profile® soil amendment. Profile Products LLC. 750 Lake Cook Road, Suite 440, Buffalo Grove, IL 60089.

[2] Amicarbazone 70 DF herbicide, Arysta LifeScience Corp., 15401 Weston Parkway Suite 150, Cary, NC 27513

[3] Trimmit® 2SC plant growth regulator, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27409.

[4] Induce®, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[5] Tee Jet® Spraying Systems Co. Wheaton, IL 60189.

[6] OS1-FL® Modulated chlorophyll fluorometer, Opti-Sciences, Inc., 8 Winn Ave., Hudson, NH 03051.

[7] Sigma Scan Pro®, Systat Software, Inc., 1735 Technology Dr., Suite 430, San Jose, CA 95110.

[8] SAS® version 9.1, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513.

Literature Cited

Anonymous, 2011. AWIS Weather Services, Inc.: Alabama Mesonet Weather Data. http://www.awis.com/forms/dasta.alawonda.html. Accessed: February 2, 2011.

Beard, J. B. 1973. Turfgrass: Science and Culture. Englewood Cliffs, NJ, Prentice-Hall. Pp. 68-69.

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

Dernoeden, P. H. 2000. Creeping Bentgrass Management: Summer Stresses, Weeds and Selected Maladies. Chelsea, MI: Sleeping Bear Press. Pp. 1-19.

Frans, R., R. Talbert, D. Marx, and H. Crowley. 1986. Experimental design and techniques for measuring and analyzing plant response to weed control practices. p. 37–38. *In* N. D. Camper (ed.), Research Methods in Weed Science, 3rd ed., Southern Weed Sci. Soc., Champaign, IL.

Genty, B., J-M, Briantais, and N. R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta. 990:87-92.

Isgrigg, J., III, F. H. Yelverton, and H. D. Coble. 1998. The effect of paclobutrazol on the relative growth of annual bluegrass and creeping bentgrass. Proc. South. Weed Sci. Soc. 51:248.

Johnson, B. J. and T. R. Murphy. 1995. Effect of paclobutrazol and flurprimidol on suppression of *Poa annua* spp. *reptans* in creeping bentgrass (*Agrostis stolonifera*) greens. Weed Technol. 9:182-186.

Karcher, D. E. and M. D. Richardson. 2005. Batch analysis of digital images to evaluate turfgrass characteristics. Crop Sci. 45:1536-1539.

Krause, G. H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:313-349.

Lycan D. W. and S. E. Hart. 2006. Seasonal effects on annual bluegrass (*Poa annua*) control in creeping bentgrass with bispyribac-sodium. Weed Technol. 20:722-727.

Maxwell, K. and G. N. Johnson. 2000. Chlorophyll fluorescence-a practical guide. J. of Exp. Bot. 51:659-668.

McCullough, P. E., S. E. Hart, D. Weisenberger, and Z. J. Reicher. 2010. Amicarbazone efficacy on annual bluegrass and saftety on cool-season turfgrasses. Weed Technol. 24:461-470.

McCullough, P. E. and S. E. Hart. 2009. Creeping bentgrass (*Agrostis stolonifera*) putting green tolerance to bispyribac-sodium. Weed Technol. 23:425-430.

McCullough, P. E. and S. E. Hart. 2006. Temperature influences creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*) response to bispyribac-sodium. Weed Technol. 20:728-732.

McDonald, S. J., Dernoeden, P. H., and Kaminski, J. E. 2006. Creeping bentgrass tolerance and annual bluegrass control with bispyribac-sodium tank-mixed with iron and nitrogen. Online. Applied Turfgrass Science doi:10.1094/ATS-2006-0811-01-RS.

Phillbrook, B. D., M. Kremer, K. H. Mueller, and R. Deege. 1999. BAY MKH 3586 – a new herbicide for broad spectrum weed control in corn (maize) and sugar cane. Brighton Conference. Weeds. 1:29-34.

Senseman, S. A. 2007. Herbicide Handbook. Lawrence, KS: Weed Science Society of America. Pp. 128-129.

Teuton, T. C., C. L. Main, J. C. Sorochan, J. S. McElroy, and T. C. Mueller. 2007. Annual bluegrass (*Poa annua*) control in creeping bentgrass (*Agrostis stolonifera*) putting greens with bispyribac-sodium. Weed Technol. 21:426-430.

Tyystjarvi, E., A. Koski, M. Keranen, and O. Nevalainen. 1999. The Kautsky curve is a built-in-barcode. Biophys. Jour. 77:1159-1167

Warren, L. S., F. H. Yelverton, and T. W. Gannon. 2009. The effect of various rates and timings of amicarbazone on bentgrass cultivar tolerance and annual bluegrass control. Proc. of South. Weed Sci. Soc. 62:386.

Yelverton, F. 2008. Spring transition from perennial ryegrass to bermudagrass and *Poa annua* management. Online. http://www.turffiles.ncsu.edu/PDFFiles/004633/Southern_California_Golf_Course_Superintendents_Association_Manhattan_Beach_CA.pdf.

Table 1. Description of study sites and parameters evaluated.

Study	Location	Species evaluated	Parameters measured
Oct 2007	Knoxville Municipal Golf Course (Knoxville, TN)	POAAN[a]	Quantum yield
Oct 2007	East TN Research and Education Center (Knoxville, TN)	AGSST	Quantum yield, herb. injury, creeping bentgrass cover
April 2008	Auburn University Turfgrass Research Unit (Auburn, AL)	POANN	Quantum yield
Nov 2008	Grand National Golf Course (Opelika, AL)	AGSST	Quantum yield, herb. injury, creeping bentgrass cover
April 2009	Grand National Golf Course (Opelika, AL)	AGSST	Quantum yield, herb. injury, creeping bentgrass cover

[a] Abbreviations: POAAN = *Poa annua*, AGSST = *Agrostis stolonifera*.

Table 2. Quantum yield of annual bluegrass following herbicide treatment expressed as a percentage relative to the nontreated.

		Fall 2007			Spring 2008		
		Weeks after treatment					
Treatment[a]	Rate (kg ai ha^{-1})	2	4	6	2	4	6
		Φ_{PSII}[b] (% relative to nontreated)					
Amicarbazone	0.10	26	20	6	31	15	10
Amicarbazone	0.20	43	24	7	43	28	40
Amicarbazone	0.30	53	29	9	56	30	56
Amicarbazone	0.40	56	34	12	74	54	63
Paclobutrazol	0.140	6	1	2	12	0	0
Paclobutrazol	0.280	4	2	0	12	0	0
LSD ($\alpha = 0.05$)		8.3	6.7	3.5	10.7	9.6	6.3

[a] Amicarbazone = Amicarbazone® 70DF Herbicide; paclobutrazol = Trimmit® 2SC Plant Growth Regulator.

[b] Symbols: Φ_{PSII} = quantum yield of photosystem II.

Table 3. Creeping bentgrass reduction in Φ_{PSII} following amicarbazone and paclobutrazol applications.

		Fall[a]			Spring 2009		
		Weeks after treatment					
Treatment[b]	Rate (kg ai ha^{-1})	2	4	6	2	4	6
		Φ_{PSII}[c] (% reduction)					
Amicarbazone	0.10	21	13	4	0	1	3
Amicarbazone	0.20	38	20	8	-6[d]	0	0
Amicarbazone	0.30	45	24	12	-8	3	4
Amicarbazone	0.40	54	33	13	-5	2	9
Paclobutrazol	0.140	4	8	2	-6	4	8
Paclobutrazol	0.280	0	3	3	-1	2	4
LSD ($\alpha = 0.05$)		6.7	4.3	3.9	5.0	4.5	3.1

[a] Data were pooled for fall 2007 and fall 2008 data.

[b] Amicarbazone = Amicarbazone® 70DF Herbicide; paclobutrazol = Trimmit® 2SC Plant Growth Regulator.

[c] Symbols: Φ_{PSII} = quantum yield of photosystem II.

[d] Negative values indicate an increase in Φ_{PSII}.

Table 4. Creeping bentgrass injury following amicarbazone and paclobutrazol applications.

		Fall[a]			Spring 2009		
		Weeks after treatment					
Treatment[b]	Rate (kg ai ha^{-1})	2	4	6	2	4	6
		% injury					
Amicarbazone	0.10	4	1	0	5	0	0
Amicarbazone	0.20	8	6	3	10	0	0
Amicarbazone	0.30	33	18	8	20	5	4
Amicarbazone	0.40	44	33	16	21	11	8
Paclobutrazol	0.140	1	5	6	0	0	0
Paclobutrazol	0.280	1	3	11	0	0	0
Nontreated	--	0	0	0	0	0	0
LSD (α = 0.05)		7.4	4.2	4.3	5.7	3.4	3.8

[a] Data were pooled for fall 2007 and fall 2008 data.

[b] Amicarbazone = Amicarbazone® 70DF Herbicide; paclobutrazol = Trimmit® 2SC Plant Growth Regulator.

[c] Creeping bentgrass injury was rated visually on a 0-100% scale, where 0 equaled no injury, 100 equaled complete death of bentgrass, and 20% was deemed an acceptable level of injury.

Table 5. Creeping bentgrass cover following amicarbazone and paclobutrazol applications.

		Fall[a]			Spring 2009		
		Weeks after treatment					
Treatment[b]	Rate (kg ai ha^{-1})	2	4	6	2	4	6
		% cover					
Amicarbazone	0.10	99	99	98	99	98	99
Amicarbazone	0.20	91	97	97	93	97	98
Amicarbazone	0.30	79	92	94	84	91	94
Amicarbazone	0.40	68	82	90	79	88	94
Paclobutrazol	0.140	99	97	92	99	98	99
Paclobutrazol	0.280	99	98	90	97	98	99
Nontreated	--	100	99	98	99	99	100
LSD ($\alpha = 0.05$)		5.1	2.6	4.6	3.1	3.5	2.9

[a] Data were pooled for fall 2007 and fall 2008 data.

[b] Amicarbazone = Amicarbazone® 70DF Herbicide; paclobutrazol = Trimmit® 2SC Plant Growth Regulator.

[c] Creeping bentgrass cover was determined using digital image analysis and is represented by a 0-100% scale, where 0 equaled no bentgrass coverage and 100 equaled complete bentgrass coverage.

Figure 2. Maximum daily temperatures in Knoxville, Tennessee and Auburn, AL in October-November 2007 and April-May 2008, respectively. Crossbars denote day of herbicide treatments for the fall 2007 and spring 2008 studies (Anonymous 2011).

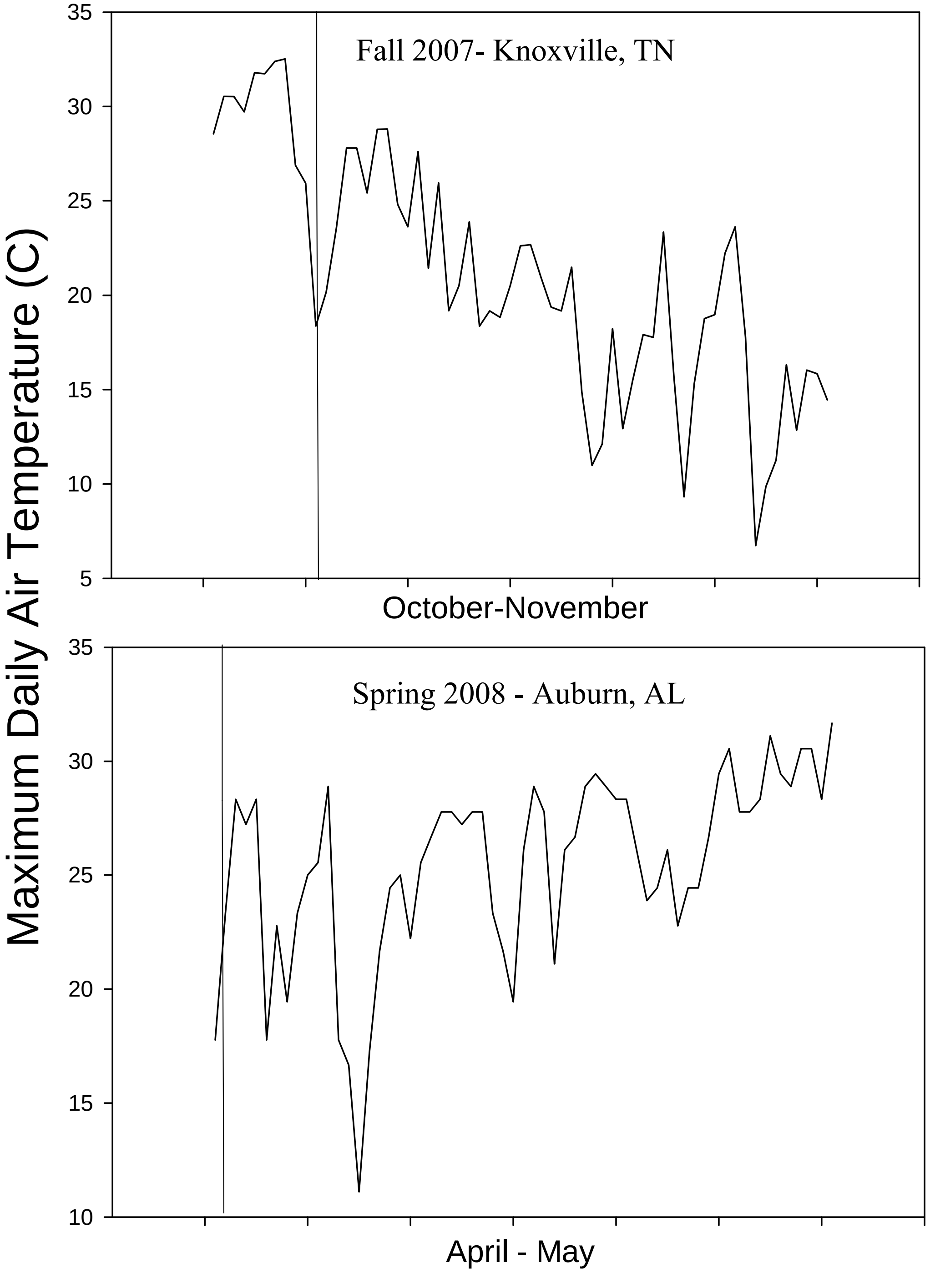


Figure 3. Annual bluegrass quantum yield following amicarbazone and paclobutrazol applications in the fall 2007 and spring 2008 studies.

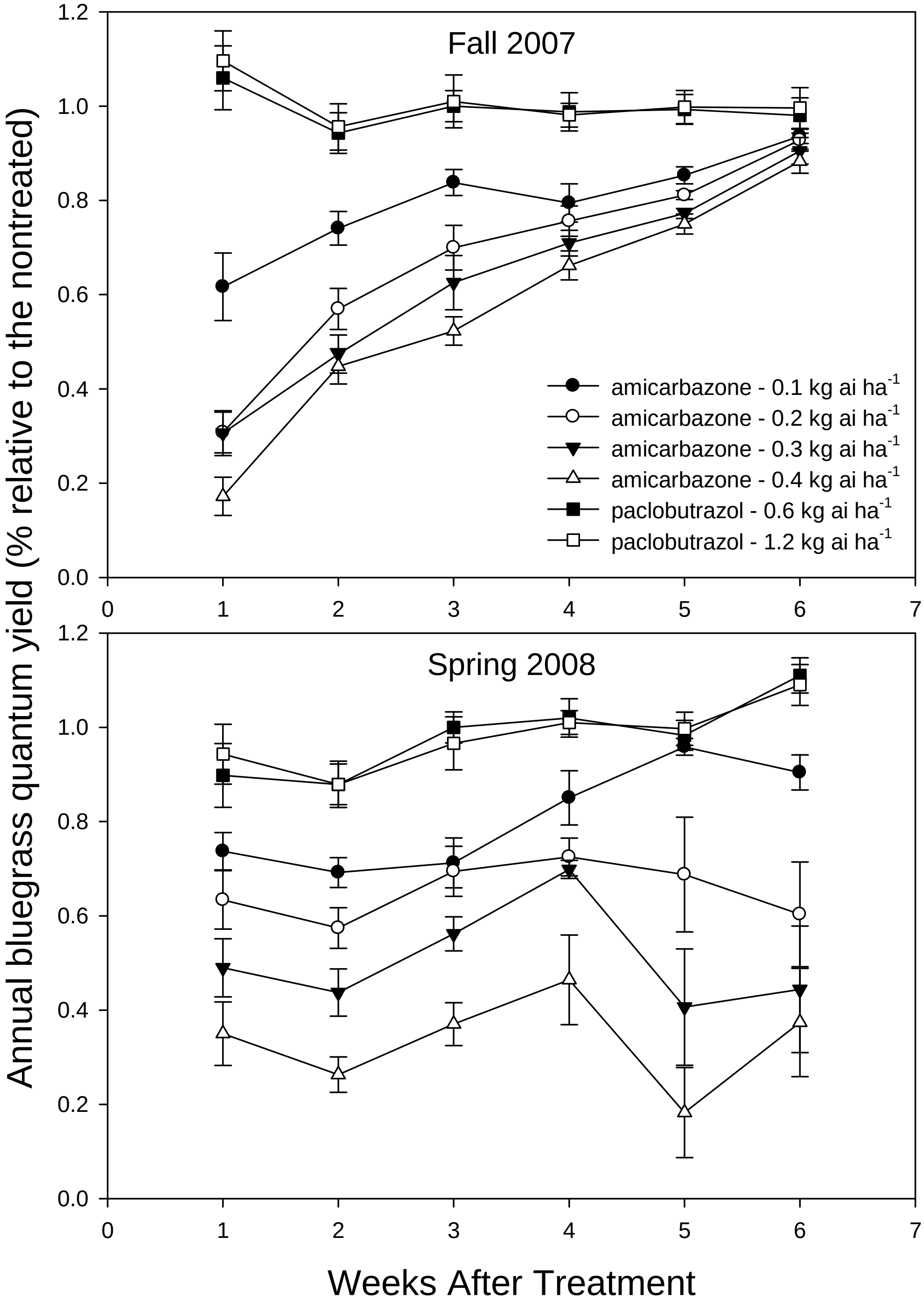
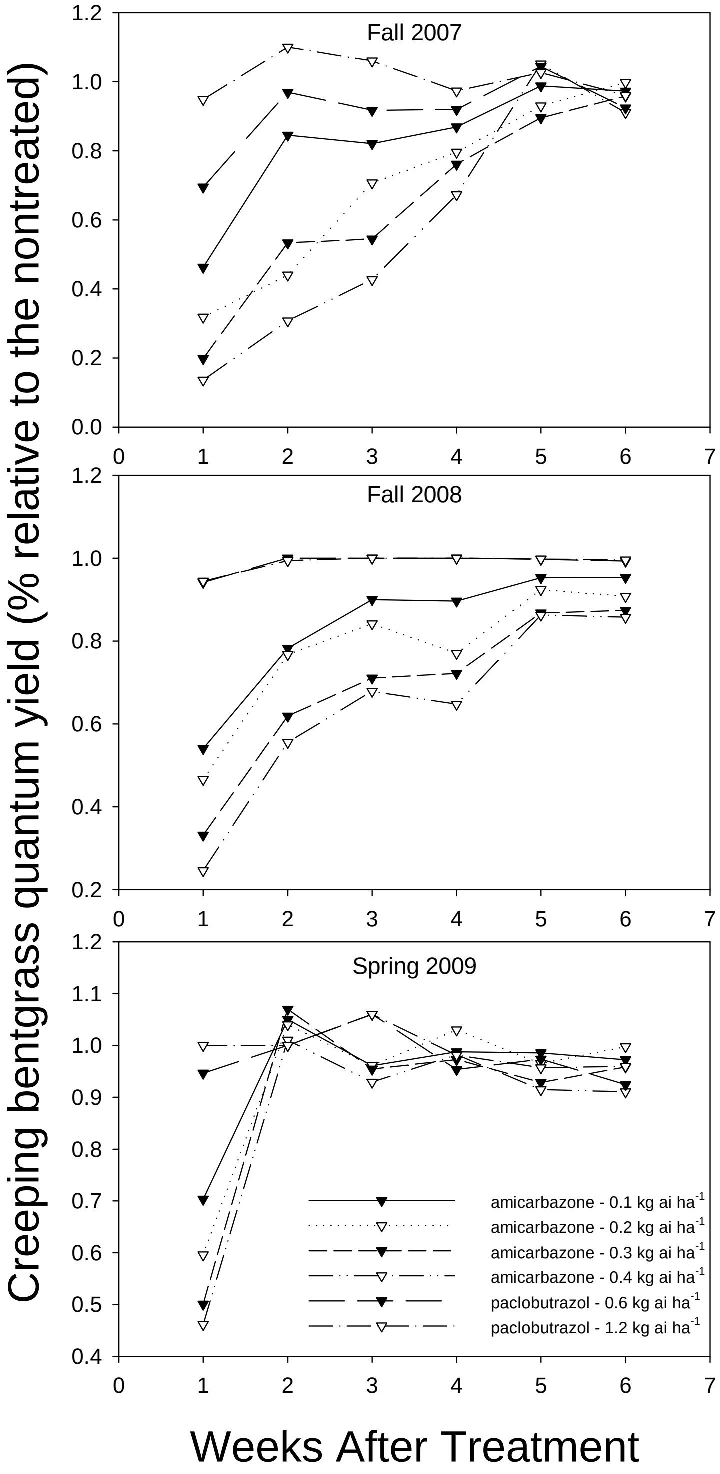


Figure 4. Creeping bentgrass quantum yield following amicarbazone and paclobutrazol applications in the fall 2007, fall 2008, and spring 2009 studies.



III. Effects of Soil vs. Foliar Application of Amicarbazone on Annual Bluegrass

Abstract

Amicarbazone is a new photosystem II (PSII) inhibiting herbicide of the triazolinone herbicide family. Greenhouse experiments were conducted to compare the effects of amicarbazone and atrazine on annual bluegrass control and quantum yield (Φ_{PSII}) when applied at three treatment placements (soil-only, foliage-only, and foliage + soil). Herbicide rates for amicarbazone and atrazine were 0.53 and 2.25 kg ha^{-1}, respectively. Amicarbazone applied soil-only and foliage + soil controlled annual bluegrass 57 and 59%, respectively 1 week after treatment (WAT). Atrazine applied to foliage + soil controlled annual bluegrass 48% 1 WAT. All soil-only and foliage + soil treatments were similar 2 WAT. Foliage-only application of amicarbazone provided significantly less control than other amicarbazone treatments. Amicarbazone applied soil-only and foliage + soil controlled annual bluegrass 100% 3 WAT. Soil-only and foliage + soil applications of atrazine and amicarbazone had similar reductions in quantum yield (Φ_{PSII}) at 1-3 WAT. Foliar-applied amicarbazone reduced Φ_{PSII} 78, 84, and 86% at 1, 2, and 3 WAT, respectively. The rapid reduction in annual bluegrass Φ_{PSII} and the increase in control resulting from soil contact of amicarbazone indicated that root exposure to amicarbazone was beneficial for annual bluegrass control.

Introduction

Amicarbazone [4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] is a triazolinone herbicide similar in activity to atrazine. Its uses include PRE and POST applications in sugar cane (*Saccharum officinarum* L.) and preplant or PRE in corn (*Zea mays* L.) (Senseman 2007). Weeds controlled include velvetleaf (*Abutilon theophrasti* Medik), common lambsquarters (*Chenopodium album* L.), and marmaladegrass [*Brachiaria plantaginea* (Link) Hitch.] (Philbrook et al. 1999; Senseman 2007). Symptoms of affected plants include chlorosis, stunted growth, tissue necrosis, and eventual death (Dayan et al. 2009; Senseman 2007).

Annual bluegrass (*Poa annua* L.) is a problematic cool-season weed species recognized by its disruption of turfgrass uniformity through differences in leaf color and copious seedhead production (Beard 1973). It has the ability to tolerate low mowing heights and compete aggressively with putting green turf. Recent studies have investigated annual bluegrass control with amicarbazone in creeping bentgrass (*Agrostis stolonifera* L.), perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.) turfgrass (Belcher and Walker 2009; McCullough et al. 2010; Walker and Belcher 2009; Warren et al. 2009). Temperature studies suggest that amicarbazone removal of annual bluegrass from cool-season turfgrass may be limited to spring applications (McCullough et al. 2010). They also reported increased amicarbazone injury and decreased clipping yields of several cool-season grass species as temperatures increased.

Amicarbazone controls susceptible weeds by inhibiting photosystem II (PSII) of (Dayan et al. 2009). Due to its lower use rates, it has been deemed a more potent PSII inhibitor than atrazine (Dayan et al. 2009; Philbrook et al. 1999). PSII-inhibiting herbicides inhibit photosynthesis by binding to the Q_B-binding niche on the D1 protein of the PSII complex. After binding on the D1 protein, electron transport from Q_A to Q_B is blocked which stops CO_2 fixation and ATP and $NADPH_2$ production. The combination of peroxidation and photolysis of thylakoid membranes and the production of singlet oxygen ultimately lead to plant death (Devine et al. 1993; Rutherford and Krieger-Liszkay 2001).

Amicarbazone like many PSII inhibitors exhibits foliar and root activity, and primary movement of amicarbazone into target plants is reportedly through plant roots (Senseman 2007). Amicarbazone effects on annual bluegrass control or photosynthesis when applied at different placements (e.g soil and/or foliar) is unknown at this time. Direct soil contact with pesticides in turfgrass systems may not always be possible as a result of thatch accumulation or herbicide formulation (McCarty 2005; Monaco et al. 2002); therefore, investigation of root versus foliar exposure of amicarbazone for annual bluegrass control is warranted. The objectives of this study were: 1) to investigate annual bluegrass control following foliage-only, soil-only, and foliage + soil selective amicarbazone placement, and 2) to evaluate annual bluegrass quantum yield (Φ_{PSII}) to better determine the effects of amicarbazone on the photosynthesis of annual bluegrass.

Materials and Methods

Establishment of Annual Bluegrass. Three greenhouse experiments were conducted in winter 2009 and spring 2010 at the Auburn University Plant Science Research Center, Auburn, AL to investigate treatment placement effects on amicarbazone efficacy. Annual bluegrass seed were harvested in 2008 from a local population and stored at 4 C prior to utilization in this study. Seed were sown in 10 cm^2 plastic pots and allowed to grow for 3 weeks. Seedlings were then individually transplanted to separate 10 cm^2 plastic pots at the 2 to 3 leaf stage. Soil medium was 90:10 (v/v) Wickam sandy loam : potting mix[1]. Following transplanting, plants were fertilized once per week with a mixture of 5 g of 24-8-16[2] per liter of water and misted daily with approximately 10 mL of water per pot. Plants were clipped weekly with hand pruning shears and allowed to grow to the 3-4 tiller stage. Greenhouse day/night temperatures were approximately 24/20 C and natural lighting was the only light source.

Evaluating the Effect of Amicarbazone and Atrazine on Annual Bluegrass Control. Treatment placements included soil-only, foliage-only, and foliage + soil applications of amicarbazone[3] at 0.53 kg ai ha^{-1} and atrazine[4] at 2.25 kg ai ha^{-1} plus 0.25% v/v nonionic surfactant[5]. Herbicides were applied on February 8, 2009 and April 1, 2010. Foliage-only treatment was accomplished by temporarily covering the soil surface with 1 cm of a granular carrier[6] to intercept any herbicide which passed through the canopy and was removed after the foliage was dry. Foliage + soil application was achieved by a normal broadcast spray application. Soil-only treatments were applied by diluting the appropriate rate of herbicide in 10 mL of water and delivering it to the soil surface with a plastic syringe avoiding contact with the foliage. Foliage-only and foliage + soil applications were applied in an enclosed spray chamber at 280 L ha^{-1} with a single 8002E nozzle[7]. Plants were not irrigated within 24 h of herbicide treatment. Annual bluegrass clipping ceased 2 weeks prior to herbicide application. The experimental designs were randomized complete blocks with six replications and blocks were rotated weekly to reduce variation in the greenhouse microclimate. Each annual bluegrass plant served as an experimental unit. Individual plants were rated for control 1, 2, and 3 WAT on a percentage scale where 0 equaled no chlorosis or necrosis, and 100 equaled plant death (Frans et al. 1986). Confirmation of normality was determined for each experiment using residual plots in PROC GLIMMIX of SAS®[8]. Data were subjected to PROC GLM in SAS® and means were separated using Fisher's Protected Least Significant Difference (LSD) test at the 0.05 significance level. Pairwise contrasts were used when making preplanned comparisons according to initial experimental objectives. Treatment by experiment interaction was not detected, therefore, experiments were combined.

Evaluating the Effects of Amicarbazone and Atrazine on Annual Bluegrass Quantum Yield. It is important to understand the basic principles governing chlorophyll fluorescence in order to create a better understanding of Φ_{PSII} and how it is affected by PSII-inhibiting herbicides. Absorption of a photon by the ground state (lowest energy) of chlorophyll results in the transition of chlorophyll to an excited (higher energy) state at which time energy is transferred to PSII reaction centers (Rohacek and Bartak 1999). Following excitation chlorophyll is unstable; therefore it gives up some energy to its surroundings in the form of heat. Following this release of heat, chlorophyll returns to a ground state. This process is very quick (several nanoseconds) and energy movement is very rapid (Taiz and Zeiger 2006). In the lowest excited state, the released energy has four pathways in which it can move: 1) it can be converted into heat, 2) energy transfer to another molecule, 3) utilization in photochemistry, or 4) re-emitted as fluorescence (Taiz and Zeiger 2006). Maxwell and Johnson (2000) and Rohacek and Bartak (1999) provide a similar list of pathways excluding step two mentioned above. These processes occur in competition with one another, therefore an increase in the efficiency of one pathway decreases the yield of the remaining processes. When PSII-inhibiting herbicides, like amicarbazone, block electron transport, the excitation energy is diverted away from photosynthetic processes and emitted as chlorophyll fluorescence and the other fates listed above.Quantum yield is calculated by the formula:

$$\Phi_{\mathrm{PSII}} = (F'_m - F_t)/F'_m$$

where F'_m equals maximum fluorescence in lighted conditions and F_t equals steady state yield of fluorescence in the light. Quantum yield measures the proportion of light absorbed by chlorophyll associated with PSII that is utilized in photosynthesis (Genty et al. 1989; Maxwell and Johnson 2000). Genty et al. (1989) considered Φ_{PSII} to be the best parameter to measure efficiency of PSII.

Quantum yield was measured 1, 2, and 3 WAT utilizing a pulse-modulated chlorophyll fluorometer[9]. Three Φ_{PSII} measurements were recorded weekly for each plant by holding the light probe approximately 45° directly above the leaf surface. Measurements were taken in mid-morning when natural lighting was available. Saturation pulse width and modulation intensity were set to 0.8 s and 6, respectively. If an annual bluegrass plant was completely controlled, the experimental unit was designated a Φ_{PSII} of 0. Φ_{PSII} measurements were converted to a percent reduction of the non-treated for each rating date using the following equation:

$$\% \, \Phi_{\mathrm{PSII}} = [(\mathrm{PY}_{\mathrm{NT}} - \mathrm{PY}_{\mathrm{T}})/ \mathrm{PY}_{\mathrm{NT}}] \times 100$$

where PY_{T} equaled Φ_{PSII} of treated annual bluegrass, and $\mathrm{PY}_{\mathrm{NT}}$ equaled Φ_{PSII} of non-treated turf. Confirmation of normality was determined for each experiment using residual plots in PROC GLIMMIX of SAS®. Data were subjected to PROC GLM in SAS® and means were separated using Fisher's Protected Least Significant Difference (LSD) test at the 0.05 significance level. Pairwise contrasts were used when making preplanned comparisons according to initial experimental objectives. Treatment by experiment interaction was not detected, therefore, experiments were combined.

Results and Discussion

Evaluating the Effect of Amicarbazone and Atrazine on Annual Bluegrass Control. Amicarbazone injury to annual bluegrass was observed as a water-soaked appearance on the distal portions of the leaves 5 DAA and symptoms progressed into chlorosis and necrosis. Symptoms of the treated plants were comparable to other PSII inhibiting herbicides (Senseman 2007). Control was influenced by herbicide 1 WAT and herbicide placement 1-3 WAT ($p < 0.01$ in all cases). No herbicide by herbicide placement interaction influenced annual bluegrass control in this study. Amicarbazone applied to foliage + soil controlled annual bluegrass 59% 1 WAT, and was similar to amicarbazone applied soil-only (Table 6). Atrazine applied to foliage + soil controlled annual bluegrass 48% 1 WAT. Amicarbazone and atrazine applied foliage-only injured annual bluegrass 30 and 34% 1 WAT, respectively. All soil-only and foliage + soil applications were similar 2 WAT. Complete control of annual bluegrass with soil-only and foliage + soil applications in conjunction with significantly less control by foliage-only application indicates that root exposure to amicarbazone was beneficial for annual bluegrass control. Negrisoli et al. (2007) observed greater weed control in sugarcane when amicarbazone was applied directly to the soil or was washed through the sugarcane straw. Although control was similar for amicarbazone and atrazine at 2 and 3 WAT, amicarbazone initially controlled annual bluegrass more than atrazine. Dayan et al. (2009) reported superior control of velvetleaf (*Abutilon theophrasti* Medik.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] with amicarbazone compared to atrazine based on biomass measurements. Treatment contrasts indicated that amicarbazone performed more effectively when soil contact was achieved (Table 7). Foliage-only applications controlled annual bluegrass 93 and 90% for amicarbazone and atrazine, respectively 3 WAT; indicating that amicarbazone was readily absorbed through the foliage of annual bluegrass and satisfactory control was achieved.

Evaluating the Effects of Amicarbazone and Atrazine on Annual Bluegrass Quantum Yield. Amicarbazone induced chlorophyll fluorescence in annual bluegrass resulting in decreased Φ_{PSII}. Similar inductions were observed by Dayan et al. (2009) following root and foliar exposure to amicarbazone. This is of primary importance since the level of chlorophyll fluorescence emitted from plant tissue gives a small insight into the photosynthetic activity of the plant in question (Krause and Weis 1991; Tyystjarvi et al. 1999). Annual bluegrass Φ_{PSII} was influenced by herbicide placement ($p < 0.001$) throughout the study, but herbicide and herbicide by placement interaction was observed 2 WAT only. Amicarbazone and atrazine applied soil-only and foliage + soil reduced Φ_{PSII} between 97 and 92% 1 WAT (Table 8). Foliage-only applications of amicarbazone and atrazine reduced Φ_{PSII} 78 and 80% 1 WAT, respectively. Complete reduction in Φ_{PSII} with soil only and foliage +soil applications of amicarbazone was observed 3 WAT while foliage-only application reduced Φ_{PSII} 86%. Rapid reduction in Φ_{PSII} with amicarbazone in this study is consistent with similar research in which foliar applications of amicarbazone nearly completely inhibited photosynthesis within 8 h after application (Dayan et al. 2009). Rapid induction of chlorophyll fluorescence from amicarbazone in this study is similar to those observed in other plant species with other PSII-inhibiting herbicides (Ali and Machado 1981; Habash et al. 1985).

Results of this study clearly demonstrate that amicarbazone was quick and efficient at inhibiting photosynthesis through foliar or root exposure (Table 9). Depending on the tolerance of certain turfgrass species to amicarbazone, inhibition of PSII may allow for certain turfgrass species to outcompete annual bluegrass. This is of particular importance on creeping bentgrass putting greens where some biotypes of annual bluegrass are able to compete with the desired turfgrass under normal maintenance conditions (Beard 1973). Currently, no registered herbicides are available that provide satisfactory POST annual bluegrass control without causing significant injury to creeping bentgrass putting greens.

Implications for Annual Bluegrass Control. Annual bluegrass can be controlled with PRE and POST herbicides in overseeded and non-overseeded bermudagrass (*Cynodon dactylon* (L.) Pers.); however, annual bluegrass control is difficult in perennial ryegrass overseed and few control options are available (McCarty 2005). Ongoing research suggests amicarbazone's primary niche is annual bluegrass control in creeping bentgrass and bermudagrass overseeded with perennial ryegrass.

The purpose of this study was to develop a better understanding of amicarbazone exposure to annual bluegrass roots and/or foliage that may be utilized in developing better annual bluegrass control strategies. This study demonstrates that satisfactory annual bluegrass control and PSII inhibition can be achieved through foliar and root exposure to amicarbazone; however, root exposure appears to be most beneficial for annual bluegrass control. This implies strategies to expose annual bluegrass roots to amicarbazone may increase control of annual bluegrass. If treated turf has an extensive thatch layer, methods to move the herbicide into the soil may need to be employed for greater annual bluegrass control. Granular herbicides, for instance, have a greater capacity than broadcast-applied liquids to pass through plant residues (Monaco et al. 2002). By describing the visual and physiological effects resulting from amicarbazone placement studies, we can better develop amicarbazone use strategies.

Amicarbazone induces annual bluegrass injury faster than atrazine, indicating that it is an efficient inhibitor of PSII. Atrazine has been researched extensively and its effects on photosynthesis are well-documented (Ali and Machado 1981; Maertens et al. 2004; Viator et al. 2002). Therefore the findings in this and previous studies indicate amicarbazone decreases photosynthesis of susceptible plants similar to atrazine. In conclusion, amicarbazone has the potential to provide POST annual bluegrass control, but factors such as selectivity in certain turf species, environmental conditions affecting herbicide activity, and plant growth stage at the time of treatment should be investigated to better understand the potential use of amicarbazone.

Sources of Materials

[1] Fafard potting mix, Conrad Fafard Inc., P.O. Box 790, Agawam, MA 01001.

[2] Miracle Grow Plant Food®, The Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, Ohio 43041.

[3] Amicarbazone® 70 DF herbicide, Arysta LifeScience Corp., 15401 Weston Parkway Suite 150, Cary, NC 27513.

[4] Aatrex® 4L herbicide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[5] Induce®, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[6] Biodac®, Kandant Grantek Inc., One Technology Park Drive, Westford, MA 01886.

[7] Tee Jet® Spraying Systems Co. Wheaton, IL 60189-7900.

[8] SAS version 9.1, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

[9] OS1-FL® Modulated chlorophyll fluorometer, Opti-Sciences, Inc., 8 Winn Ave., Hudson, NH 03051.

Literature cited

Ali, A. and V. S. Machado. 1981. Rapid detection of ‘triazine resistant’ weeds using chlorophyll fluorescence. Weed Res. 21:191-197.

Beard, J. B. 1973. Turfgrass: Science and Culture. Englewood Cliffs, NJ: Prentice-Hall. Pp. 67-68.

Belcher, J. and R. Walker. 2009. Annual weed control with amicarbazone and flucarbazone in warm-season turf. Proc. of South. Weed Sci. Soc. 62:190.

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

Devine, M., S. O. Duke, and C. Fedtke. 1993. Physiology of Herbicide Action. New Jersey: Prentice Hall. Pp. 113-136.

Frans, R., R. Talbert, D. Marx, and H. Crowley. 1986. Experimental design and techniques for measuring and analyzing plant response to weed control practices. p. 37–38. *In* N. D. Camper (ed.), Research Methods in Weed Science, 3rd ed., Southern Weed Sci. Soc., Champaign, IL.

Genty, B., J-M, Briantais, and N. R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta. 990:87-92.

Habash, D., M. P. Percival, and N. R. Baker. 1985. Rapid chlorophyll fluorescence technique for the study of penetration of photosynthetically active herbicides into leaf tissue. Weed Res. 25:389-395.

Krause, G. H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The Basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:313-349.

Maertens, K. D., C. L. Sprague, P. J. Tranel, and R. A. Hines. 2004. *Amaranthus hybridus* populations resistant to triazine and acetolactase synthase-inhibiting herbicides. Weed Res. 44:21-26.

Maxwell, K. and G. N. Johnson. 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51:659-668.

McCarty, L. B. 2005. Best Golf Course Management Practices. 2nd ed, Upper Saddle River, NJ: Pearson Prentice Hall.

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.

Monaco, T. J., S. C. Weller, and F. M. Ashton. 2002. Weed Science Principles and Practices 4th ed. New York: John Wiley & Sons, Inc. Pp. 78-79, 148.

Negrisoli, E., C. V. S. Rossi, E. E. Velini, a. L. Cavenaghi, E. A. D. Costa, and R. E. B. Toledo. 2007. Weed control by amicarbazone applied in the presence of sugar-cane straw. Planta Daninha. 25:603-611.

Philbrook, B. D., M. Kremer, K. H. Mueller, and R. Deege. 1999. BAY MKH 3586 – a new herbicide for broad spectrum weed control in corn (maize) and sugarcane. Brighton Conference. Weeds. 1:29-34.

Rohacek, K. and M. Bartak. 1999. Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. Photosynthetica. 37:339-363.

Rutherford, A. W. and A. Krieger-Liszkay. 2001. Herbicide-induced oxidative stress in photosystem II. Trends in Biochem. Sci. 26:648-653.

Senseman, S. A. 2007. Herbicide Handbook. Lawrence, KS: Weed Science Society of America. Pp. 128-129.

Taiz, L. and E. Zeiger. 2006. Plant Physiology 4th ed. Sunderland, MA: Sinauer Associates, Inc., Publishers. Pp. 125-158.

Tyystjarvi, E., A. Koski, M. Keranen, and O. Nevalainen. 1999. The Kautsky curve is a built-in-barcode. Biophys. Jour. 77:1159-1167.

Viator, B. J., J. L. Griffin, and E. P. Richard, Jr. 2002. Evaluation of red morningglory (*Ipomoea coccinea*) for potential atrazine resistance. Weed Technol. 16:96-101.

Walker, R. H. and J. L. Belcher. 2009. Annual bluegrass control with amicarbazone in perennial ryegrass overseed. Proc. of South. Weed Sci. Soc. 62:191.

Warren, L. S., F. H. Yelverton, and T. W. Gannon. 2009. The effect of various rates and timings of amicarbazone on bentgrass cultivar tolerance and annual bluegrass control. Proc. of South. Weed Sci. Soc. 62:386.

Table 6. Annual bluegrass injury or control following herbicide applications pooled over three greenhouse experiments.

Treatment	Rate	Annual bluegrass injury/control[a]		
		1 WAT[b]	2 WAT	3 WAT
	(kg ai ha^{-1})	----------------------%----------------------		
Non-treated		0	0	0
Foliage-only				
Amicarbazone	0.53	34	85	93
Atrazine	2.25	30	89	90
Soil-only				
Amicarbazone	0.53	57	99	100
Atrazine	2.25	55	96	97
Foliage + soil				
Amicarbazone	0.53	59	97	100
Atrazine	2.25	48	98	100
LSD (0.05)		6.5	3.4	4.8

[a] Injury was rated on a 0-100% scale where 0 = no injury and 100 = complete control.

[b] Abbreviations: WAT = Weeks After Treatment.

Table 7. Treatment contrasts of annual bluegrass control following foliage, soil, and foliage + soil applications of amicarbazone and atrazine pooled over three greenhouse experiments.

	Annual bluegrass control		
	------------Pr > F-------------		
Treatment contrasts	1 WAT[a]	2 WAT	3 WAT
Soil versus foliar applied amicarbazone	<.0001[b]	<.0001	0.0164
Soil versus soil + foliage applied amicarbazone	0.6781	0.2796	1.0000
Foliar versus soil + foliar applied amicarbazone	<.0001	<.0001	0.0164
Amicarbazone versus atrazine applied to soil only	0.4471	0.1381	0.2856
Amicarbazone versus atrazine applied to foliage only	0.3337	0.0269	0.3314
Amicarbazone versus atrazine applied to soil + foliage	0.0066	0.4984	1.0000

[a] Abbreviation: WAT = Weeks After Treatment.

[b] Values < 0.05 are statistically significant.

Table 8. Decrease in annual bluegrass quantum yield following herbicide applications pooled over three greenhouse experiments.

		Annual bluegrass Φ_{PSII}		
Treatment	Rate	1 WAT[b]	2 WAT	3 WAT
	(kg ai ha^{-1})	--------------------------%--------------------------		
Non-treated		0[c]	0	0
Foliage-only				
Amicarbazone	0.53	78	84	86
Atrazine	2.25	80	71	80
Soil-only				
Amicarbazone	0.53	97	99	100
Atrazine	2.25	92	93	96
Foliage + soil				
Amicarbazone	0.53	96	100	100
Atrazine	2.25	93	100	100
LSD (0.05)		5	6	7

[a] Φ_{PSII} = Quantum yield of photosystem II. Reduction values were calculated as a percentage of the nontreated.

[b] Abbreviations: WAT = Weeks After Treatment.

Table 9. Contrasts of quantum yield of annual bluegrass following foliage, soil, and foliage + soil applications of amicarbazone and atrazine pooled over three greenhouse experiments.

	Annual bluegrass Φ_{PSII}[a]		
	--------------Pr > F--------------		
Treatment contrasts	1 WAT[b]	2 WAT	3 WAT
Soil versus foliar applied amicarbazone	<.0001[c]	<.0001	0.0046
Soil versus soil + foliage applied amicarbazone	0.5178	0.7843	0.7716
Foliar versus soil + foliar applied amicarbazone	<.0001	<.0001	<.0001
Amicarbazone versus atrazine applied to soil only	0.0722	0.0523	0.3850
Amicarbazone versus atrazine applied to foliage only	0.6650	<.0001	0.1066
Amicarbazone versus atrazine applied to soil + foliage	0.3231	0.9478	1.000

[a] Φ_{PSII} = Quantum yield of photosystem II. Reduction values were calculated as a percentage of the nontreated.

[b] Abbreviation: WAT = Weeks After Treatment.

[c] Values < 0.05 are statistically significant.

IV. Cool-Season Turfgrass Response to Amicarbazone as Affected by Temperature and Application Timing on Perennial Ryegrass Overseed

Abstract

Amicarbazone is a photosystem II (PSII)-inhibiting herbicide being evaluated for annual bluegrass control in bermudagrass overseeded with perennial ryegrass. Growth chamber experiments were conducted to investigate the effects of temperature on perennial ryegrass and annual bluegrass response to amicarbazone. Amicarbazone was applied at 0, 0.13, 0.26, or 0.52 kg ai ha^{-1} and bispyribac-sodium (bispyribac) at 0.07 kg ai ha^{-1} was included as a standard treatment. Herbicides were applied to annual bluegrass and perennial ryegrass growing in two temperature regimes: 24/12 C day/night or 14/4 C day/night. As temperatures increased from 14/4 C to 24/12 C and amicarbazone rate increased, perennial ryegrass and annual bluegrass injury increased. Quantum yield of annual bluegrass and perennial ryegrass decreased 50-80% compared to non-treated levels within 24 h of application. This suggests PSII of both species was sensitive to amicarbazone regardless of temperature. Perennial ryegrass clipping yields and turf color decreased greater than annual bluegrass in both temperature regimes 2 and 4 WAT. Two field experiments were conducted in 2008-2010 to evaluate amicarbazone application timing effects on annual bluegrass control and perennial ryegrass response in bermudagrass overseeded with perennial ryegrass. Herbicides were applied at 0.26 or 0.52 kg ha^{-1} at 1, 2, 4, 8, or 16 WAO. In the field studies, amicarbazone applied at 0.26 or

0.52 kg ha^{-1} 1 to 4 WAO injured perennial ryegrass $\geq$ 64%4 WAT. The 8 WAO applications injured perennial ryegrass 71-89% and 4-11% in 2009 and 2010, respectively 4 WAT. Amicarbazone applied at 0.26 and 0.52 kg ha^{-1} 16 WAO was safe on perennial ryegrass in both studies. Amicarbazone applied at 0.52 kg ha^{-1} 16 WAO controlled annual bluegrass 79 and 86% in 2009 and 2010, respectively. These data indicate that temperature and amicarbazone rate significantly affected amicarbazone activity on perennial ryegrass overseed and annual bluegrass. In addition, amicarbazone applied 16 WAO provided the best combination of perennial ryegrass safety and annual bluegrass control.

Introduction

Bermudagrass is a warm-season turfgrass utilized in various aspects of golf course, athletic field, and home lawn turf. Bermudagrass growth slows in the fall as temperatures decrease, and dormancy can be observed if temperatures drop below 10 C for an extended period of time (Beard 1973; McCarty 2005). Perennial ryegrass is a cool-season turfgrass species commonly utilized in overseeding warm-season turfgrass (Horgan and Yelverton 2001). Overseeding is an agronomic practice which involves seeding into an existing warm-season turfgrass stand in order to enhance the aesthetics and/or playability of the turf during bermudagrass winter dormancy (Puhalla et al. 1999). Perennial ryegrass has a dark-green color, fine texture, and waxy leaf surface which all contribute to the desirable striping characteristics observed on athletic fields and golf courses (Puhalla et al. 1999).

Annual bluegrass is a ubiquitous, cool-season grass species which commonly plagues fine turf. The light-green mottling observed during severe infestations in conjunction with its competition for nutrients, moisture, and light makes annual bluegrass undesirable for turf practitioners. In the South, the primary germination period for annual bluegrass is in the fall, followed by a secondary germination period in the spring. Vargas and Turgeon (2004) concluded that annual bluegrass can germinate any time adequate moisture is available, but other factors such as pH, light, and nutrients may impact germination. Annual bluegrass flowering and panicle production begins in late-winter to early spring and continues throughout favorable growing conditions (Wells 1974). A single annual bluegrass plant can produce hundreds of seeds which promote future infestations (Lush 1989). Panicles are light-green to white in color and drastically disrupt the aesthetic quality of the desired turf (Lycan and Hart 2006). In non-overseeded bermudagrass turf, annual bluegrass is free of turfgrass competition; therefore annual bluegrass plants may become large and unsightly.

PRE and POST herbicides are available to practitioners for utilization in non-overseeded situations (Toler et al. 2007). PRE herbicides such as prodiamine or pendimethalin are applied in late summer to prevent germination of annual bluegrass (Bhowmik and Bingham 1990; Dernoeden 1998). Several sulfonylurea herbicides such as rimsulfuron, trifloxysulfuron, and foramsulfuron provide good POST annual bluegrass control in non-overseeded turf (Brecke et al. 2005). In overseeding situations, however, few POST herbicide options are available that provide annual bluegrass control and good perennial ryegrass safety.

Bispyribac is registered for POST annual bluegrass control in bermudagrass overseeded with perennial ryegrass (Anonymous 2008). Temperature is reported to affect bispyribac activity on annual bluegrass (McCullough and Hart 2006). Practitioners are cautioned not to apply bispyribac when temperatures are below 13 C and not expected to exceed 13 C within 3 DAA. Optimum annual bluegrass control with minimum perennial ryegrass injury is expected with sunny conditions and daytime maximum air temperatures between 21 and 27 C (Anonymous 2008). Bispyribac applied once or twice sequentially controlled annual bluegrass 82 and 94%, respectively. However, sequential applications significantly reduced perennial ryegrass cover (McElroy et al. 2011). Ethofumesate has been utilized since the early 1980's for annual bluegrass control in overseeding situations. Bermudagrass must be dormant when making ethofumesate applications or bermudagrass transition the following spring may be interrupted (Johnson 1983). Dickens (1979) observed bermudagrass injury following fall applications, but did not observe bermudagrass transition issues the following spring.

Amicarbazone is a photosystem II (PSII)-inhibiting herbicide being investigated for annual bluegrass control in bermudagrass overseeded with perennial ryegrass. McCullough et al. (2010) reported increased injury to perennial ryegrass and annual bluegrass with amicarbazone as temperatures increased from 10 to 30 C. Injury was exacerbated with sequential applications. Walker and Belcher (2009) reported near complete annual bluegrass control and minimal perennial ryegrass injury with spring amicarbazone applications.

Research is needed to better understand the susceptibility of various cool-season grass species to amicarbazone. The objectives of this research were 1) to evaluate annual bluegrass and perennial ryegrass physiological and phenotypic response to single amicarbazone applications while grown in different temperature regimes and 2) to evaluate perennial ryegrass response and annual bluegrass control to different amicarbazone application timings.

Materials and Methods

Growth Chamber Study. Two growth chamber[5] experiments were conducted in spring 2010 to evaluate perennial ryegrass and annual bluegrass response to amicarbazone under different temperature regimes. Both studies were conducted at the Auburn University Plant Science Research Center in Auburn, AL. ‘Goalkeeper’ perennial ryegrass was seeded at 896 kg ha^{-1} and annual bluegrass was seeded at 45 kg ha^{-1} into 10.2 cm^2 plastic pots. The annual bluegrass population utilized in this study was characterized by wide leaf blades and an upright growth habit. Soil medium was 90:10 (v/v) Wickham sandy loam : potting mix[6] (pH – 6.0). Pots were watered daily until both species were established at which time pots were watered as needed to prevent wilting. Plants were fertilized once per week with 5 g of 24-8-16[7] L^{-1} of water and hand-clipped at 2.5 cm until treatments were initiated. Plants were grown for 8 weeks under greenhouse conditions and allowed to acclimate in their respective growth chambers for 1 week prior to herbicide treatment. One growth chamber was programmed for 14/4 C day/night conditions while the other was programmed for 24/12 C day/night conditions. Both growth chambers emitted 500 μmol m^{-2} s^{-1} 12 h per day.

Herbicide treatments included single applications of amicarbazone at 0, 0.13, 0.26, or 0.52 kg ha^{-1} and bispyribac[8] at 0.07 kg ha^{-1}. Herbicides were applied in an enclosed spray chamber delivering 280 L ha^{-1} with an 8002E nozzle. The turfgrass was not clipped or irrigated within 24 hours before or after application.

Visual injury was rated on a percentage scale (0-100), where 0 equaled no chlorosis or necrosis and 100 equaled complete death of perennial ryegrass. Perennial ryegrass cover was rated on a percentage scale, where 0 = no perennial ryegrass and 100 equaled complete perennial ryegrass coverage. Turf quality was rated on a 0-9 scale with 0 equal to dead turf and 9 equal to optimum turf (Toubakaris and McCarty 2000). Quantum yield (Φ_{PSII}) was measured utilizing a pulse-modulated chlorophyll fluorometer[9]. Three yield measurements were recorded for each pot by holding the light probe at approximately 45° directly above the turf canopy. The saturation pulse width and modulation intensity were set to 0.8 s and 6, respectively. Green color was determined using an NDVI (normalized difference vegetation index) meter[10]. All measurements and ratings were conducted 2 and 4 WAT. Clipping yields were collected at 2 and 4 WAT by removing all foliage 2.5 cm above the soil surface. Clippings were oven-dried at 62 C for 72 h and weighed.

Pots were organized in a split block design with four replications within each chamber. The study was analyzed as a factorial arrangement with five herbicides treatments by two temperatures by two turfgrass species. An additional factor for HAA was included for quantum yield data. Data were subjected to normality testing using residual plots in PROC GLIMMIX of SAS (Littell et al. 2006). Fixed effects included herbicide, temperature, turfgrass species, and interactions of these factors. Random effects included trial and block (trial × temperature), turfgrass species × block (trial × temperature, turfgrass species × herbicide (trial × temperature), and HAA × block (trial

× temperature). This model was chosen based on the smallest AICC value output by the PROC GLIMMIX fit statistics. Main effects and interactions were subjected to analysis of variance in PROC GLIMMIX using type III tests of fixed effects at $\alpha = 0.05$. Trials were not significantly different for quantum yield data; therefore, experimental data was pooled across both growth chamber experiments. Treatment contrasts for turfgrass injury were conducted for preplanned comparisons according to original experimental objectives.

Field Study. Two field experiments were conducted at the Auburn University Turfgrass Research Unit (TGRU) from October 2008 to April 2009 and from October 2009 to April 2010 to evaluate annual bluegrass control and perennial ryegrass overseed response following different amicarbazone application rates and timings. The soil was a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludults). Both studies were conducted on 'Tifway' hybrid bermudagrass overseeded with 'PhD' perennial ryegrass. Study sites were overseeded on October 22, 2008 and October 15, 2009 at 896 kg ha^{-1}. Plots were fertilized with 24 kg N ha^{-1} mo^{-1} and mown at 2.5 cm weekly in the fall and winter and twice weekly in the spring. Amicarbazone[1] was applied at 0.26 or 0.53 kg ai ha^{-1} at 1, 2, 4, 8, or 16 WAO. A nonionic surfactant[2] was added to all herbicide mixtures at 0.25% v/v. A non-treated check was included in both studies. Herbicides were applied to 1.5 by 1.5 m plots with a CO_2 pressurized backpack sprayer using 8002VS nozzles[3] calibrated to deliver 280 L ha^{-1} of spray solution at 138 kPa. All experimental plots were visually evaluated for perennial ryegrass injury on a percentage scale, where 0 equaled no chlorosis or necrosis and 100 equaled complete death of perennial ryegrass. Perennial ryegrass cover was rated on a percentage scale, where 100 equaled complete perennial ryegrass cover and 0 equaled no perennial ryegrass. Perennial ryegrass quality was rated on a 0-9 scale, where 0 equaled worst quality and 9 equaled best quality. Annual bluegrass control was visually evaluated in the spring for each study on a percentage scale, where 0 equaled no annual bluegrass chlorosis or necrosis and 100 equaled complete death of annual bluegrass. For discussion purposes, 80% annual bluegrass control was considered a satisfactory level of control (Frans and Talbert 1977).

Treatments (Table 10) were organized in randomized complete blocks with four replications. Treatments were a factorial combination of amicarbazone rate by application timing. Data structure and normality was analyzed using residual plots in PROC GLIMMIX of SAS[4]. Fixed effects included amicarbazone rate, application timing, and amicarbazone rate by application timing while random effects included trial, block, and block (trial). This model was chosen based on the smallest AICC value output by the PROC GLIMMIX fit statistics. Main effects and interactions were subjected to analysis of variance using type III tests of fixed effects in PROC GLIMMIX at $\alpha = 0.05$. Perennial ryegrass injury, cover, and annual bluegrass control were significantly different between years for some application timings therefore data will be presented separately.

Results and Discussion

Growth Chamber Study. Turfgrass injury was first observed as a minor chlorosis and progressed over time to leaf tip necrosis. Amicarbazone applied at 0.52 kg ha^{-1} killed some perennial ryegrass plants. A herbicide by temperature by turfgrass species interaction ($P < 0.001$) was detected 2 WAT. Herbicide by temperature and herbicide by turfgrass species were significant ($P < 0.001$) throughout the study. Annual bluegrass and perennial ryegrass injury increased with increasing amicarbazone rates at 14/4 and 24/12 C. McCullough et al. (2010) observed similar results with amicarbazone. Amicarbazone applied at 0.26 and 0.52 kg ha^{-1} injured perennial ryegrass greater than annual bluegrass regardless of temperature. This observation differed from similar growth chamber studies which observed slightly safer activity on perennial ryegrass compared to annual bluegrass (McCullough et al. 2010). Amicarbazone applied at 0.13 kg ha^{-1} injured annual bluegrass and perennial ryegrass < 5% at the 14/4 C temperature regime (Table 11). Amicarbazone applied at 0.52 kg ha^{-1} at 14/4 C injured annual bluegrass and perennial ryegrass 18 and 59%, respectively 4 WAT. Amicarbazone applied at 0.52 kg ha^{-1} at 24/12 C injured annual bluegrass and perennial ryegrass 36 and 84%, respectively 4 WAT. Bispyribac injured perennial ryegrass 30 and 29% at 2 and 4 WAT, respectively, at 24/12 C. Bispyribac injured annual bluegrass similar to amicarbazone at 14/4 C. Amicarbazone at 0.26 kg ha^{-1} injured annual bluegrass and perennial ryegrass 11-13 and 24-35% at 14/4-24/12 C, respectively, 4 WAT.

A herbicide by temperature by turfgrass species interaction ($P < 0.001$) was detected 4 WAT only. A herbicide by turfgrass species and herbicide by temperature interaction was detected 2 and 4 WAT. Relative NDVI of amicarbazone-treated annual bluegrass was > 96% of non-treated levels at 14/4 C 2 and 4 WAT (Table 12). Amicarbazone applied at 0.52 kg ha^{-1} at 14/4 reduced perennial ryegrass relative NDVI >35% 4 WAT. Perennial ryegrass NDVI at 14/4 C was >93% of non-treated levels for all other herbicide treatments. Amicarbazone applied at 0.52 kg ha^{-1} at 24/12 C reduced annual bluegrass NDVI >20% 2 WAT, but recovered to 93% WAT. Amicarbazone applied at 0.52 kg ha^{-1} at 24/12 C reduced perennial ryegrass NDVI >25% 2 WAT, but minimal recovery was observed 4 WAT. Bispyribac applied at 0.07 kg ha^{-1} reduced relative NDVI the greatest on perennial ryegrass at 24/12 C 2 WAT. All other bispyribac treatments reduced NDVI <7% relative to the non-treated.

A significant amicarbazone rate by temperature ($P = 0.046$) and amicarbazone rate by turfgrass species ($P < 0.001$) interaction was detected for clipping yield data 2 WAT. No amicarbazone rate by temperature by turfgrass species interaction was detected throughout the study. Clipping yields decreased as amicarbazone rates increased (Table 13). In general, amicarbazone reduced clipping yields of perennial ryegrass greater than annual bluegrass. Amicarbazone applied at 0.13 kg ha^{-1} at 14/4 C resulted in a hormesis effect on both grass species (Figure 5). Amicarbazone applied at 0.52 kg ha^{-1} at 14/4 C to annual bluegrass and perennial ryegrass reduced clipping yield 16 and 62%, respectively 2 WAT. At 24/12 C, perennial ryegrass clipping yields were reduced greater than annual bluegrass at all amicarbazone rates 2 and 4 WAT. Amicarbazone applied at 0.52 kg ha^{-1} at 24/12 C reduced annual bluegrass clipping yield greater than all amicarbazone rate/temperature combinations. Amicarbazone applied at 0.26 and 0.52 kg ha^{-1} at 14/4 C reduced annual bluegrass clipping yields 7 and 43%, respectively 4 WAT. At the same rates and temperature, perennial ryegrass clipping yields were reduced 63 and 92% 4 WAT. Amicarbazone applied at 0.26 and 0.52 kg ha^{-1} at 24/12 C reduced annual bluegrass clipping yields 13 and 59%, respectively 4 WAT. At the same rates and temperature, perennial ryegrass clipping yields were reduced 55 and 84%, respectively 4 WAT. A similar trend in perennial ryegrass clipping yield reduction with amicarbazone has been previously reported (McCullough et al. 2010).

A significant herbicide by temperature by turfgrass species by HAA interaction ($P < 0.001$) was detected for quantum yield data. Amicarbazone reduced quantum yield >50% 24 HAA, regardless of rate, turfgrass species, or temperature regime. Amicarbazone applied at 0.52 kg ha^{-1} at 24/12 C to perennial ryegrass caused the greatest initial reduction in quantum yield. A similar trend in PSII recovery was detected for both turfgrass species; however, greater recovery was observed for annual bluegrass (Figure 6). Photosystem II recovery was greatest with annual bluegrass at 24/12 C, although some residual effects of amicarbazone activity were still observed. Amicarbazone applied to perennial ryegrass at 0.52 kg ha^{-1} reduced quantum yield > 80 4 WAT, indicating increased perennial ryegrass sensitivity at that rate and temperature.

Injury, NDVI, clipping yield, and quantum yield data indicate that annual bluegrass was more tolerant of amicarbazone than perennial ryegrass at the growth stage tested. This may be due to differences in sensitivity at the growth stage of the turf species utilized in this study. Good annual bluegrass control and perennial ryegrass safety has been reported in perennial ryegrass with similar amicarbazone rates used in this study; however, previous applications were made to mature perennial ryegrass in the spring (Walker and Belcher, 2009). Annual bluegrass tolerance decreased as temperatures and amicarbazone rate increased, and perennial ryegrass was more sensitive to amicarbazone than annual bluegrass in this scenario.

Field Study. Perennial ryegrass injury was visible as a general chlorosis of treated plants. Significant ($P < 0.001$) main effects and interactions were detected at 2 and 4 WAT for both the 2008 and 2009 study. In 2008, amicarbazone applied at 0.26 kg ha^{-1} at 1-8 WAO injured perennial ryegrass >59% 2 WAT (Table 14). Amicarbazone applied at 0.52 kg ha^{-}

^{1} at 1-8 WAO injured perennial ryegrass >75% 2 WAT. Perennial ryegrass treated 1-8 WAO was injured >64% 4 WAT. Acceptable injury was observed on perennial ryegrass treated 16 WAO with no injury being observed 4 WAT.

In 2009, similar perennial ryegrass injury was observed on plots treated 1-4 WAO. The 8 WAO applications were significantly different from the 2008 study. Amicarbazone applied at 0.26 and 0.52 kg ha^{-1} 8 WAO injured perennial ryegrass 10 and 14%, respectively 2 WAT. Perennial ryegrass treated 8 WAO recovered to 4 and 11% injury, respectively 4 WAT. Amicarbazone applied at 0.26 and 0.52 kg ha^{-1} 16 WAO injured perennial ryegrass 6 and 13%, respectively 2 WAT. Amicarbazone applications at 1-4 WAO caused severe chlorosis and significant perennial ryegrass death. Ryegrass death was also observed with 8 WAO applications in 2008 only.

Amicarbazone applied 1-8 WAO in 2008 reduced perennial ryegrass cover to unacceptable levels in spring 2009 (Table 15). Amicarbazone applied at 0.52 kg ha^{-1} 8 WAO reduced perennial ryegrass cover >80%. Plots treated with amicarbazone at 0.26 and 0.52 kg ha^{-1} 16 WAO had 90 and 88% perennial ryegrass cover.

In 2009, amicarbazone applied 1 to 4 WAO reduced perennial ryegrass cover >35% for all application timings except amicarbazone applied at 0.26 kg ha^{-1} 1 WAO. Amicarbazone applied at 0.26 kg ha^{-1} 1 WAO and 0.26 and 0.52 kg ha^{-1} 8 WAO reduced cover significantly less in spring 2010 than in spring 2009. The greater injury and reductions in perennial ryegrass cover observed with the 8 WAO applications between the two studies may be attributed to differences in temperature following amicarbazone application. Following treatment in 2008, the perennial ryegrass experienced 15 days of >19 C as opposed to the 2009 study which experienced 2 days of > 19 C (Figure 7). Our growth chamber studies and other recent amicarbazone studies have reported differences in cool-season turf phytotoxicity due to increasing temperatures from 10 to 30 C (McCullough et al. 2010). Amicarbazone applied at 0.26 kg ha^{-1} at 1 WAO reduced cover 41 and 5% in 2009 and 2010. Differences observed in turf cover for the 1 WAO applications may be attributed to the level of perennial ryegrass emergence at the time of application. Less perennial ryegrass emergence suggests less foliar exposure to amicarbazone.

Amicarbazone applied at 0.52 kg ha^{-1} controlled annual bluegrass significantly greater ($P < 0.001$) than amicarbazone at 0.26 kg ha^{-1} in 2009 and 2010 (Table 15). Amicarbazone applied 1 WAO controlled annual bluegrass 68-82%. Amicarbazone applied at 0.52 kg ha^{-1} 2 WAO controlled annual bluegrass 74% in 2009 and 2010. Amicarbazone applied at 4 and 8 WAO did not provide acceptable control of annual bluegrass. Amicarbazone applied at 0.52 kg ha^{-1} 16 WAO controlled annual bluegrass 79-86% in 2009 and 2010.

Amicarbazone applied sequentially at lower rates than those utilized in this study may successfully control annual bluegrass while maintaining perennial ryegrass safety. Creeping bentgrass is sensitive to rates utilized in this study, but sequential applications of amicarbazone at 0.10 kg ha^{-1} reportedly controls annual bluegrass while maintaining turf safety (McCullough et al. 2010; Yelverton 2008). Annual bluegrass control did not follow a consistent trend with application timing. The 8 WAO application timing for example, may be explained by drastic temperature differences between the 2 years. Some application timings in 2008 significantly reduced perennial ryegrass cover. Less annual bluegrass control observed in these plots may be due to less perennial ryegrass competition. Bingham et al. (1969) observed less annual bluegrass on putting green plots overseeded with cool-season grasses. Amicarbazone applied at 0.52 kg ha^{-1} 16 WAO caused minimal perennial ryegrass injury and acceptable annual bluegrass control. The same rate at a similar application timing controlled annual bluegrass 99% in a previous study (Walker and Belcher 2009). Walker and Belcher (2009) observed 39 and 79% annual bluegrass control with single amicarbazone applications at 0.13 kg ha^{-1} and 0.26 kg ha^{-1} in March; however, control increased to 89 and 99% when applied sequentially at the same rates. Amicarbazone applied at 0.26 kg ha^{-1} 16 WAO controlled annual bluegrass 38-43% in this research; however, our applications were made approximately 4 weeks prior to previous studies (Walker and Belcher 2009). Amicarbazone applied at 0.52 kg ha^{-1} 16 WAO in this study was the only treatment to consistently control annual bluegrass while maintaining perennial ryegrass safety. This may be due to the growth stage of annual bluegrass at the time of application. These studies suggest higher rates or sequential applications may be warranted to control larger, tillered annual bluegrass plants. Warmer temperatures in the spring may also account for an increase in annual bluegrass control. As indicated in our growth chamber studies and previous research, temperature affects amicarbazone activity (McCullough et al. 2010).

In conclusion, amicarbazone has the potential to be an incremental component of an annual bluegrass control program in perennial ryegrass overseed. Based on the results of these and previous studies, amicarbazone use on bermudagrass overseeded with perennial ryegrass should be limited to mature plants in the spring. Unacceptable perennial ryegrass injury and thinning may result from earlier applications. Although the 0.52 kg ha^{-1} amicarbazone rate was safe on perennial ryegrass overseed in these studies, lower rates applied sequentially may enhance annual bluegrass control. However, more field studies should be conducted in the spring to evaluate rates, sequential applications, and tank-mixtures for annual bluegrass control programs in perennial ryegrass overseed.

Sources of Materials

[1] Amicarbazone® 70 DF herbicide, Arysta LifeScience Corp., 15401 Weston Parkway Suite 150, Cary, NC 27513.

[2] Induce®, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[3] Tee Jet® Spraying Systems Co. Wheaton, IL 60189-7900.

[4] SAS® version 9.1, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

[5] Adaptis A1000, Conviron, 590 Berry Street, Winnipeg, Manitoba.

[6] Fafard potting mix, Conrad Fafard Inc., P.O. Box 790, Agawam, MA 01001.

[7] Miracle Grow Plant Food®, The Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, Ohio 43041.

[8] Velocity® 80SP Herbicide, Valent Professional Products, P. O. Box 8025, Walnut Creek, CA 94596.

[9] OS1-FL® Modulated chlorophyll fluorometer, Opti-Sciences, Inc., 8 Winn Ave., Hudson, NH 03051.

[10] Field Scout TCM 500 NDVI Turf Color Meter, Spectrum Technologies, Inc., 12360 S. Industrial Dr. East, Plainfield, IL 60585.

Literature Cited

Anonymous, 2011. AWIS Weather Services, Inc.: Alabama Mesonet Weather Data. http://www.awis.com/forms/dasta.alawonda.html. Accessed: February 2, 2011.

Anonymous. 2008. Velocity SG Herbicide. Valent U.S.A. Corporation. Walnut Creek, CA 94596.

Beard, J. B. 1973. Turfgrass: Science and Culture. Prentice Hall. New Jersey.

Bhowmik, P. C. and S. W. Bingham. 1990. Preemergence activity of dinitroaniline herbicides used for weed control in cool-season turfgrasses. Weed Technol. 4:387–393.

Bingham, S. W., R. E. Schmidt, and C. K. Curry. 1969. Annual bluegrass control in overseeded bermudagrass putting green turf. Agron. J. 61:908-911.

Brecke, B., D. O. Stephenson, and J. B. Unruh. 2005. Annual bluegrass control in overseeded bermudagrass. ASA-CSSA-SSSA International Meeting. No. 64-9.

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

Dickens, R. 1979. Control of annual bluegrass (*Poa annua*) in overseeded bermudagrass (*Cynodon* spp.) golf greens. Weed Sci. 27:642-644.

Frans, R. E., and R. E. Talbert. 1977. Design of field experiments and the measurement of plant responses. Pp. 18-19. *In* B. Truelove, Research Methods in Weed Science, 2nd ed., Southern Weed Sci. Soc., Auburn, AL.

Horgan, B. P. and F. H. Yelverton. 2001. Removal of perennial ryegrass from overseeded bermudagrass using cultural methods. Crop Sci. 41:118–126.

Johnson, B. J. 1983. Response to ethofumesate of annual bluegrass (*Poa annua*) and overseeded bermudagrass (*Cynodon dactylon*). Weed Sci. 31:385-390.

Littell, R. C, G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models 2nd ed. SAS Publishing. Cary, N.C.

Lush, W. M. 1989. Adaptation and differentiation of golf course populations of annual bluegrass (*Poa annua*). Weed Sci. 37:54-59.

Lycan, D. W. and S. E. Hart. 2006. Seasonal effects of annual bluegrass (*Poa annua*) control in creeping bentgrass with bispyribac-sodium. Weed Technol. 20:722-727.

McCarty, L. B. 2005. Best Golf Course Management Practices. 2nd ed. Pearson Prentice Hall. New Jersey.

McCullough, P. E., S. E. Hart, D. Weisenberger, and Z. J. Reicher. 2010. Amicarbazone efficacy on annual bluegrass and saftety on cool-season turfgrasses. Weed Technol. 24:461-470.

McCullough, P. E., and S. E. Hart. 2006. Temperature influences creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*) response to bispyribac-sodium. Weed Technol. 20:728-732.

McElroy, J. S., G. K. Breeden, and G. Wehtje. 2011. Evaluation of annual bluegrass control programs for bermudagrass turf overseeded with perennial ryegrass. Weed Technol. 25:58-63.

Puhalla, J., J. Krans, and M. Goatley. 1999. Sports Fields: A Manual For Design Construction and Maintenance. John Wiley & Sons, Inc. New Jersey. Pp. 24-26, 269.

Toler, J. E., T. G. Willis, A. G. Estes, and L. B. McCarty. 2007. Postemergent annual bluegrass control in dormant nonoverseeded bermudagrass turf. HortSci. 42:670-672.

Toubakaris, M. and B. McCarty. 2000. Heat stress separates old and new bentgrasses: Cultivars vary in their responses to summertime stress. Golf Course Manage. 68:49-53.

Vargas, Jr., J. M., and A. J. Turgeon. 2004. *Poa Annua*: Physiology, Culture, and Control of Annual Bluegrass. John Wiley & Sons, Inc. New Jersey.

Walker, R. H. and J. L. Belcher. 2009. Annual bluegrass control with amicarbazone in perennial ryegrass overseed. Proc. of South. Weed Sci. Soc. 62:191.

Wells, G. J. 1974. The biology of *Poa annua* and its significance in grassland. Herbage Abstr. 44:385-391.

Yelverton, F. 2008. Spring transition from perennial ryegrass to bermudagrass and *Poa annua* management. Online. http://www.turffiles.ncsu.edu/PDFFiles/004633/Southern_California_Golf_Course_Superintendents_Association_Manhattan_Beach_CA.pdf.

Table 10. Treatment structure and perennial ryegrass growth stage of application timing studies.

Herbicide[a]	Rate kg ai ha^{-1}	Application timing WAO	Ryegrass growth stage[c]
Amicarbazone	0.26	1[b]	1 leaf
Amicarbazone	0.52	1	1 leaf
Amicarbazone	0.26	2	2 leaf
Amicarbazone	0.52	2	2 leaf
Amicarbazone	0.26	4	3-4 leaf
Amicarbazone	0.52	4	3-4 leaf
Amicarbazone	0.26	8	≈7 leaf
Amicarbazone	0.52	8	≈7 leaf
Amicarbazone	0.26	16	> 4 tiller
Amicarbazone	0.52	16	> 4 tiller

[a] Amicarbazone = Amicarbazone 70 DF Herbicide.

[b] Abbreviations: WAO = Weeks After Overseeding.

[c] Perennial ryegrass growth stage represents an approximation of surveyed plants prior to herbicide treatment.

Table 11. Pooled treatment means and pairwise contrasts[a] conducted on preplanned comparisons for annual bluegrass and perennial ryegrass injury following amicarbazone and bispyribac-sodium applications.

Pairwise contrast	Rate ($kg\ ai\ ha^{-1}$)	2 WAT[b]	4 WAT
		———% injury———	
Amicarbazone vs. B.S[c] on AB at 14/4	0.13	2 vs. 5	4 vs. 8
Amicarbazone vs. B.S on PR at 14/4		0 vs. 18*	2 vs. 8*
Amicarbazone vs. B.S on AB at 24/12		4 vs. 8*	8 vs. 8
Amicarbazone vs. B.S on PR at 24/12		4 vs. 30*	11 vs. 29*
PR at 14/4 vs. 24/12		0 vs. 4*	2 vs. 11*
AB at 14/4 vs. 24/12		2 vs. 4	4 vs. 8
AB vs. PR at 14/4		2 vs. 0	4 vs. 2
AB vs. PR at 24/12		4 vs. 4	8 vs. 11
Amicarbazone vs. B.S on AB at 14/4	0.26	2 vs. 5	11 vs. 8
Amicarbazone vs. B.S on PR at 14/4		6 vs. 18*	24 vs. 26
Amicarbazone vs. B.S on AB at 24/12		10 vs. 8	13 vs. 8
Amicarbazone vs. B.S on PR at 24/12		47 vs. 30*	35 vs. 29*
PR at 14/4 vs. 24/12		6 vs. 47*	24 vs. 35*
AB at 14/4 vs. 24/12		2 vs. 10*	11 vs. 13
AB vs. PR at 14/4		2 vs. 6	11 vs. 24*
AB vs. PR at 24/12		10 vs. 47*	13 vs. 35*
Amicarbazone vs. B.S on AB at 14/4	0.52	6 vs. 5	18 vs. 8*
Amicarbazone vs. B.S on PR at 14/4		11 vs. 18*	59 vs. 26*
Amicarbazone vs. B.S on AB at 24/12		46 vs. 8*	36 vs. 8*
Amicarbazone vs. B.S on PR at 24/12		79 vs. 30*	84 vs. 29*
PR at 14/4 vs. 24/12		11 vs. 79*	59 vs. 84*
AB at 14/4 vs. 24/12		6 vs. 46*	18 vs. 36*
AB vs. PR at 14/4		6 vs. 11*	18 vs. 59*
AB vs. PR at 24/12		46 vs. 79*	36 vs. 84*

[a] Pooled treatment means followed by * indicate a significant difference at P = 0.05.

[b] Abbreviations: WAT = Weeks After Treatment, B.S. = bispyribac-sodium, PR = perennial ryegrass, AB = annual bluegrass.

[c] Bispyribac-sodium was applied at 0.07 kg ai ha^{-1}.

Table 12. Annual bluegrass and perennial ryegrass color response[a] following herbicide application at different temperature regimes.

			Annual bluegrass		Perennial ryegrass	
	Rate	Temperature	2 WAT[b]	4 WAT	2 WAT	4 WAT
Herbicide	kg ai ha^{-1}	(day/night C)	NDVI (% of nontreated)			
Amicarbazone	0.13	14/4	99.8	100	98.4	99
	0.26		98.6	99.5	95.6	93.3
	0.52		97.9	96.4	93.4	64.8
Bispyribac-sodium	0.07		97.4	100	93.4	94.5
Amicarbazone	0.13	24/12	98.8	95.7	99.3	98.1
	0.26		95.9	94.1	89.8	90.8
	0.52		77.8	93.6	73.2	78.3
Bispyribac-sodium	0.07		97.3	99.2	88.5	98

[a] Color response measured utilizing an NDVI turf color meter.

[b] Abbreviations: WAT = Weeks After Treatment; NDVI = normalized difference vegetative index.

Table 13. Annual bluegrass and perennial ryegrass relative clipping weights following herbicide application at different temperature regimes.

			Annual bluegrass		Perennial ryegrass	
	Rate	Temperature	2 WAT[b]	4 WAT	2 WAT	4 WAT
Herbicide[a]	kg ai ha^{-1}	(day/night C)	Relative clipping yield[c] (%)			
Amicarbazone	0.13	14/4	119[d]	107	107	119
	0.26		108	93	65	37
	0.52		84	57	38	8
Bispyribac-sodium	0.07		93	121	45	74
Amicarbazone	0.13	24/12	116	95	95	89
	0.26		92	87	49	45
	0.52		51	41	25	16
Bispyribac-sodium	0.07		83	100	45	81

[a] Amicarbazone = Amicarbazone® 70 DF herbicide; Bispyribac-sodium = Velocity® 80 SP herbicide.

[b] Abbreviations: WAT = Weeks After Treatment; NDVI = normalized difference vegetative index.

[c] Relative clipping yields were calculated as a percentage of the nontreated clipping yields.

[d] Values > 100 represent a hormesis effect (growth promotion).

Table 14. Perennial ryegrass injury following amicarbazone treatment at different application timings.

Amicarbazone[a] rate kg ai ha^{-1}	Timing WAO	2008 2 WAT[b]	2008 4 WAT	2009 2 WAT	2009 4 WAT
		% injury			
0.26	1	73	70	74	64
0.52	1	78	79	83	83
0.26	2	83	85	88	88
0.52	2	83	91	86	94
0.26	4	59	65	59	64
0.52	4	84	89	86	95
0.26	8	61	71	10	4
0.52	8	88	89	14	11
0.26	16	5	0	6	3
0.52	16	6	0	13	5
Nontreated	--	0	0	0	0

[a] Amicarbazone = Amicarbazone 70 DF herbicide.

[b] Abbreviations: WAT = Weeks After Treatment, WAO = Weeks After Overseeding.

Table 15. Perennial ryegrass cover and annual bluegrass control following amicarbazone treatment at different application timings.

Amicarbazone[c] rate kg ai ha^{-1}	Timing WAO	Perennial ryegrass[a] 2009	Perennial ryegrass[a] 2010	Annual bluegrass[b] 2009	Annual bluegrass[b] 2010
		———% cover———		———% control———	
0.26	1[d]	59	95	68	82
0.52	1	50	63	74	79
0.26	2	55	55	22	34
0.52	2	50	51	74	74
0.26	4	66	65	16	19
0.52	4	45	31	43	70
0.26	8	60	99	19	5
0.52	8	18	100	20	3
0.26	16	90	99	43	38
0.52	16	88	98	79	86
Nontreated	--	85	96	0	0

[a] Perennial ryegrass cover ratings were taken 19 WAO.

[b] Annual bluegrass control ratings were taken 23 WAO.

[c] Amicarbazone = Amicarbazone 70 DF herbicide.

[d] Abbreviations: WAO = Weeks After Overseeding.

Figure 5. Relative clipping yields of annual bluegrass and perennial ryegrass following amicarbazone application in two temperature regimes.

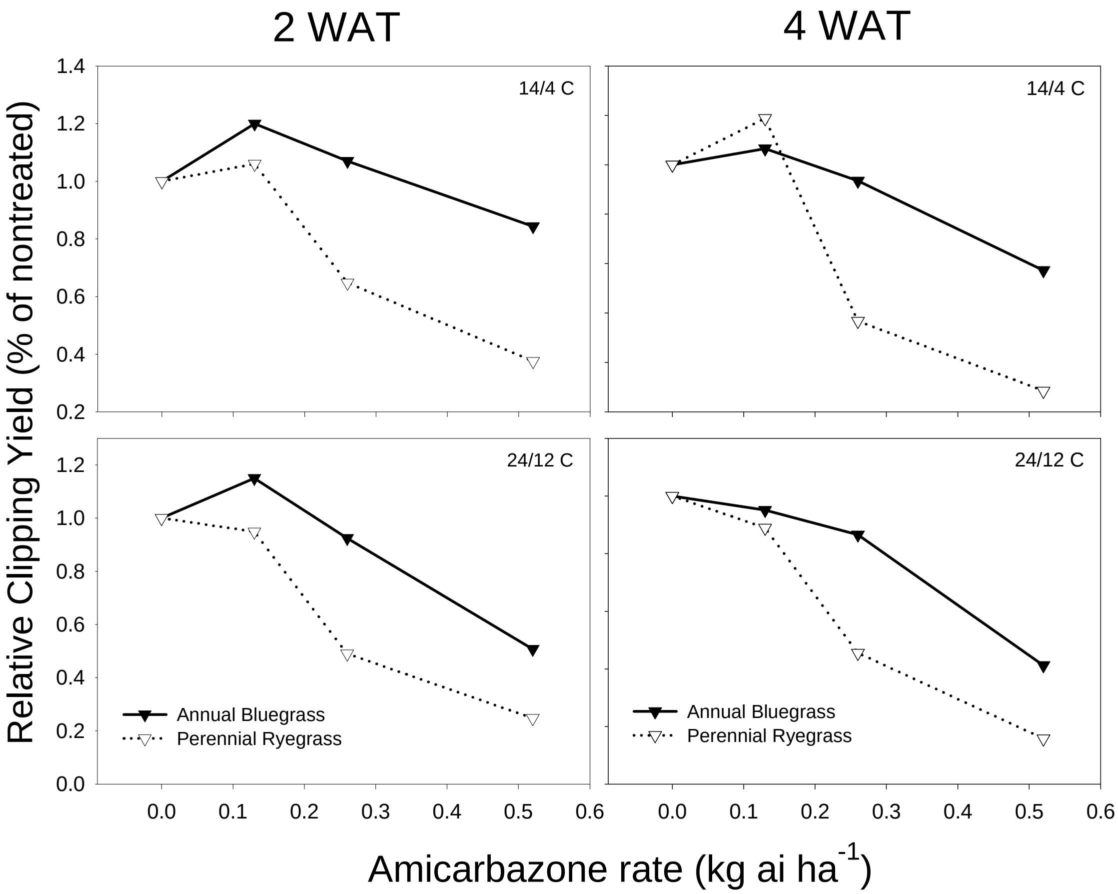


Figure 6. Relative quantum yield of annual bluegrass and perennial ryegrass following amicarbazone application in two temperature regimes.

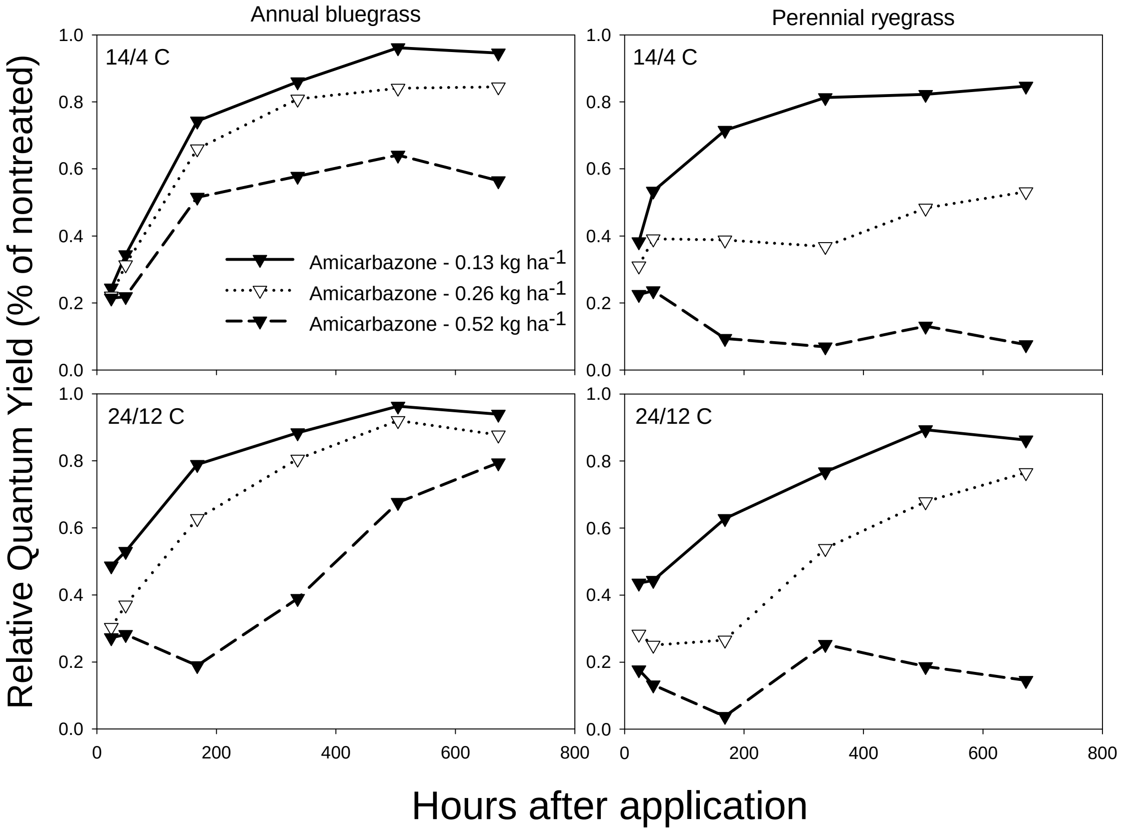
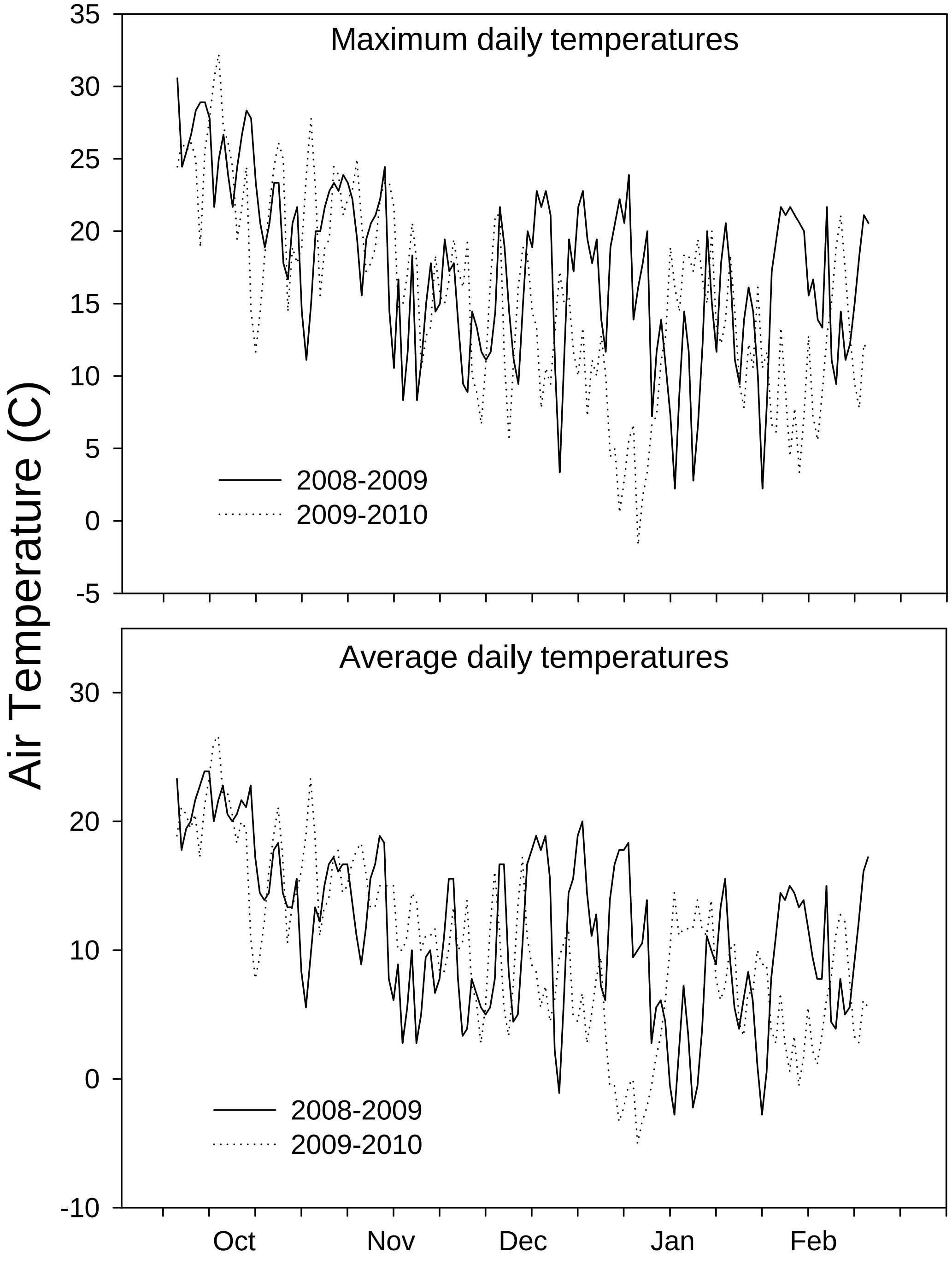


Figure 7. Maximum and average daily temperatures for the 2008 and 2009 study periods beginning October 1 and ending on February 28 (Anonymous 2011).



V. Evaluation of Triazine-Resistant Annual Bluegrass Populations for Potential Cross-Resistance to Amicarbazone

Abstract

Amicarbazone is a photosystem II (PSII)-inhibiting herbicide similar in mode of action to the triazine herbicides family. Annual bluegrass is a widely-distributed, cool-season grass species and resistance to some PSII-inhibiting herbicides has been reported. Recent research suggests amicarbazone controls annual bluegrass in various turfgrass scenarios. The objective of this study was to evaluate triazine-resistant and –susceptible annual bluegrass populations for potential cross-resistance to amicarbazone. Two triazine-resistant (MS-01, MS-02) and –susceptible (AL-01, COM-01) annual bluegrass populations were treated with amicarbazone, atrazine, and simazine at 0.26, 1.7, and 1.7 kg ai ha^{-1}, respectively. Quantum yield (Φ_{PSII}) of annual bluegrass was measured 0 to 72 hours after application (HAA) to determine the photochemical effects of amicarbazone compared to other PSII inhibitors. Neither triazine-resistant annual bluegrass population was controlled with amicarbazone. Quantum yield data of triazine-susceptible populations suggest amicarbazone efficiently inhibits PSII immediately following treatment. Amicarbazone inhibited PSII of susceptible populations significantly greater than atrazine and simazine 1 to 16 and 1 to 72 HAA, respectively. Amicarbazone did not reduce Φ_{PSII} of the MS-01 population. Amicarbazone slightly reduced Φ_{PSII} of the MS-

02 population during several measurement timings; however, these reductions were not dramatic and were not further investigated. These data indicate amicarbazone efficiently inhibited PSII of susceptible annual bluegrass populations; however, triazine-resistant annual bluegrass populations may possess cross-resistance to amicarbazone.

Introduction

Annual bluegrass is a cool-season grass species that commonly infests golf courses, athletic fields, residential, and utility turfgrass. Numerous herbicides are available that provide PRE and POST control of annual bluegrass in non-overseeded bermudagrass (Brecke et al. 2005; Toler et al. 2007). Simazine, an *s*-triazine herbicide, has been applied for years in non-overseeded turf to provide practitioners a successful, economical means of controlling annual bluegrass (Hutto et al. 2004; Yelverton and Isrigg 1998).

Triazine-resistant annual bluegrass was first reported on a highway right-of-way in Normandy, France in 1975 following 10 years of repeated simazine use (Darmency and Gasquez 1983). Triazine-resistance among annual bluegrass populations in the U.S. has been reported in Alabama, Oregon, North Carolina, Mississippi, and Virginia (Heap 2011). Kelly et al. (1999) reported a 1000-fold level of simazine resistance in annual bluegrass collected from two locations in Mississippi. Sequencing of the chloroplast psbA gene of resistant populations indicated a ser_{264} to gly mutation as commonly observed with triazine-resistant biotypes (Hirschberg and McIntosh 1983; Kelly et al. 1999; Kumata et al. 2001). Hutto et al. (2004) screened annual bluegrass from 20 golf courses in Mississippi for simazine resistance; 18 of which harbored resistant populations. Of the 18 resistant sites, simazine applied at 28.6 kg ai ha^{-1} did not control annual bluegrass in least one of three sample areas from each site (Hutto et al. 2004).

Amicarbazone is a photosystem II-inhibiting herbicide being evaluated for annual bluegrass control in fine turf (McCullough et al. 2010; Walker and Belcher 2009; Yelverton 2008). Amicarbazone applied sequentially in March at 0.13 and 0.26 kg ha^{-1} controlled annual bluegrass 89 and 99%, respectively with minimal injury to perennial ryegrass in overseeded bermudagrass (Walker and Belcher 2009). Single amicarbazone applications in March at 0.52 kg ha^{-1} yielded similar results (Walker and Belcher 2009). Amicarbazone applied sequentially at 0.1 to 0.3 kg ha^{-1} in the spring controlled annual bluegrass with minimal creeping bentgrass injury while fall applications were too injurious to creeping bentgrass (McCullough et al. 2010; Yelverton 2008).

Amicarbazone efficiently halts electron transport, inducing chlorophyll fluorescence of susceptible plants (Dayan et al. 2009). Amicarbazone's PSII-inhibiting mode of action (MOA) causes rapid death of susceptible plants; however, the potential for cross-resistance with other PS II-inhibiting herbicides is a concern (Dayan et al. 2009). Preliminary screening of triazine-resistant annual bluegrass populations with amicarbazone revealed no control (unpublished data) or symptom expression. The objective of this research was to evaluate PSII sensitivity of triazine-resistant and –susceptible annual bluegrass biotypes through Φ_{PSII} assays following amicarbazone, atrazine, and simazine applications.

Materials and Methods

Growth of Annual Bluegrass. Greenhouse studies were conducted at the Auburn University Plant Science Research Center in Auburn, AL. Four annual bluegrass populations were utilized in the study. Susceptible plants consisted of an Auburn, AL population (AL-01) and a commercial population (COM-01). Resistant plants consisted of Winona (MS-01) and Tupelo, Mississippi (MS-02) populations utilized in previous research (Hutto et al. 2004). The COM-01 population was characterized by an upright growth habit, course leaf texture, and diffuse tillering. The AL-01 susceptible population and the MS-01 resistant population were characterized by narrow leaf blades and diffuse tillering. The MS-02 resistant population was characterized by narrow leaf blades and dense tillering.

Annual bluegrass was seeded into 10 by 10 cm plastic pots. Plants were watered daily until emergence at which time pots were thinned to five plants per pot. The soil medium was 90:10 (v/v) Wickham sandy loam : potting mix[1] with 6.0 pH. Following annual bluegrass establishment, plants were watered as needed to prevent wilting, fertilized once per week with 5 g of 24-8-16[2] fertilizer L^{-1} of water, and hand-clipped at 5.0 cm until treatments were initiated. Greenhouse temperatures were approximately 22/20 C day/night throughout the study. Annual bluegrass plants were grown to a 3 to 5 tiller growth stage prior to treatment initiation. Herbicide treatments included amicarbazone[3], atrazine[4], simazine[5] applied at 0.26, 1.7, and 1.7 kg ai ha^{-1}, respectively. Herbicide solutions contained 0.25% v/v nonionic surfactant[6] and a non-treated control was included for comparison. Herbicides were applied in an enclosed spray chamber delivering 280 L ha^{-1} with an 8002E nozzle[7].

Evaluation and Ratings. Quantum yield was measured using a pulse-modulated chlorophyll fluorometer[8]. Two annual bluegrass plants comparable in growth stage were selected within each pot prior to initiation of herbicide treatments. These plants were marked with white (plant #1) and red (plant #2) stakes so repeat measurements could be taken from the same plants during each measurement timing and decrease variation among subjects. Plants were placed in artificial lighting 30 min prior to and during each measurement and placed in greenhouse growing conditions otherwise. The saturation pulse width and modulation intensity of the chlorophyll fluorometer were set to 0.8 s and 6, respectively. Measurements were taken by holding the light probe at approximately 45° directly above the desired annual bluegrass leaf blade. Two measurements were taken from mature annual bluegrass leaves for each annual bluegrass plant for a total of four measurements per pot per measurement timing. Quantum yield was measured 0, 1, 2, 4, 8, 16, 24, 48, and 72 HAA. Quantum yield data for each population were calculated as a percentage of the non-treated plants for individual measurement timing. Annual bluegrass control was rated visually on a percent scale (0-100) where 0 equaled no chlorosis or necrosis and 100 equaled complete death of annual bluegrass plants (Frans et al. 1986).

Pots were organized in a randomized complete block design with four replications and the research was repeated in time. The study was analyzed as a factorial arrangement with three herbicide treatments by four annual bluegrass populations by nine measurement timings. Annual bluegrass control data were analyzed as a factorial arrangement of three herbicide treatments by four annual bluegrass populations. Data were subjected to normality testing using residual plots in PROC GLIMMIX of SAS[9] (Littell et al. 2006). Fixed effects for Φ_{PSII} data included herbicide, annual bluegrass population, HAA, and interactions of these factors. Random effects included experimental run, block (experiment), herbicide × block (experiment), HAA × block (experiment), population × block (study), and herbicide × HAA × block (study). This model was chosen based on the smallest Akaike information correction criterion (AICC) value output by the PROC GLIMMIX fit statistics. Main effects and interactions were subjected to analysis of variance in PROC GLIMMIX using type III tests of fixed effects at $\alpha = 0.05$. Trials were not significantly different for Φ_{PSII} data; therefore, experimental data were pooled across both greenhouse experiments.

Results and Discussion

Amicarbazone symptomology was consistent with other PSII inhibitors (Senseman 2007). Symptoms of susceptible plants treated with amicarbazone first appeared as necrotic leaf-tips 4 to 5 days following treatment. Chlorosis and eventual necrosis migrated down the leaf blades as time progressed. A treatment-by-run interaction was nonsignificant ($p > 0.05$) for annual bluegrass control 1 and 2 WAT, and quantum yield of annual bluegrass; therefore, experiments were pooled for these data. Amicarbazone and atrazine controlled the COM-01 population 61 and 82%, respectively 1 week after treatment (WAT) (Table 16). Amicarbazone and atrazine controlled the AL-01 population 89 and 78%, respectively 1 WAT. Simazine controlled the COM-01 and AL-01 susceptible populations 36 and 24%, respectively 1 WAT. All herbicides controlled the triazine-susceptible biotype of annual bluegrass >94% 2 WAT. Neither resistant annual bluegrass population was controlled in this study.

It is important to understand the basic principles governing chlorophyll fluorescence in order to create a better understanding of Φ_{PSII} and how it is affected by PSII-inhibiting herbicides. Quantum yield (Φ_{PSII}) measures the amount of chlorophyll-absorbed light associated with PSII that is used in photochemistry and is considered to be the most useful parameter for measuring efficiency of PSII (Maxwell and Johnson 2000). Genty et al. (1989) defined quantum yield as:

$$\Phi_{PSII} = (F'_m - F_t)/F'_m$$

where F'_m = fluorescence maximum in a lighted state and F_t = steady-state fluorescence immediately following a flash of light (Maxwell and Johnson 2000). The accumulation of energy from continual absorption of light by chlorophyll results in an increase in the rate of chlorophyll *a* fluorescence and steady-state fluorescence as a form of energy release (Gressel 2002). In contrast, plants which exhibit a mutated target site (i.e. triazine-resistant annual bluegrass) do not exhibit this increase in fluorescence. As a result, herbicide resistance may be detected through the level of chlorophyll fluorescence emitted from a plant (Shaw et al. 1985). Utilizing chlorophyll fluorescence data to draw conclusions from plant-herbicide interactions is well documented (Shaw et al. 1985; Ali and Machado 1981; Habash et al. 1985; Menendez et al. 2006).

All fixed effects for Φ_{PSII} data were significant ($P < 0.001$). Amicarbazone quickly reduced Φ_{PSII} of both triazine-susceptible populations (Figure 8). Amicarbazone reduced Φ_{PSII} of the COM-01 population significantly greater than the triazine herbicides 1-16 HAA. Atrazine and amicarbazone reduced Φ_{PSII} of the COM-01 population similarly 24-72 HAA. Simazine reduced Φ_{PSII} of the COM-01 population significantly less than amicarbazone and atrazine 1-48 HAA. A clear separation of treatment means was observed with the AL-01 susceptible population; similar to the COM-01 susceptible population. Amicarbazone significantly reduced Φ_{PSII} compared to atrazine and simazine 1-24 and 1-72 HAA, respectively. Chlorophyll fluorescence has been utilized as a rapid technique for resistance detection (Ali and Machado 1981; Habash et al. 1985). Ali and Machado (1981) utilized rapid chlorophyll fluorescence induction as a tool to diagnose triazine-resistant redroot pigweed (*Amaranthus retroflexus* L.). Following treatment with atrazine, a 138% increase in fluorescence was observed in susceptible biotypes 2 h after treatment while no increase in fluorescence was observed in the resistant biotype.

Conversely, amicarbazone and the triazines did not reduce Φ_{PSII} of the MS-01 resistant population 0-72 HAA. Dayan et al. (2009) warned of such events following assays on triazine-resistant pigweed thylakoid membranes. Similar responses were observed with the MS-02 resistant population. Amicarbazone did reduce Φ_{PSII} <10% for some measurement timings with the MS-02 population, however, this reduction was not dramatic and did not deviate from the general trend observed in the MS-01 population.

Least square means of non-treated plants were analyzed to determine if differences in Φ_{PSII} of triazine-resistant and -susceptible biotypes existed with no herbicide pressure. Non-treated plants of the susceptible populations possessed a significantly greater Φ_{PSII} than resistant populations throughout the study (Figure 9). Triazine-resistant annual bluegrass is reported to have a reduced photosynthetic capacity and an increased sensitivity to photoinhibition compared to the susceptible biotype when grown in full-sunlight (Kumata et al. 2001). Holt (1988) observed reduced photosynthetic efficiency in triazine-resistant common groundsel (*Senecio vulgaris* L.) compared to the susceptible biotype. In addition, fitness costs in the form of reduced biomass, seed production, and leaf area among triazine-resistant weed populations is well-documented (Conard and Radosevich 1979; Holt and Radosevich 1983; Holt 1988).

In conclusion, amicarbazone is a PSII-inhibiting herbicide being evaluated in fine turf for annual bluegrass control. These data suggest amicarbazone controls the susceptible annual bluegrass biotype rapidly. Amicarbazone applied at 15% of the atrazine and simazine rate reduced Φ_{PSII} of the susceptible biotype significantly greater than atrazine and simazine indicating a more efficient PSII-inhibition. For example, amicarbazone reduced Φ_{PSII} of the COM-01 and AL-01 susceptible populations 54 and 71%, respectively 1 HAA. Conversely, triazine-resistant annual bluegrass populations were not controlled with amicarbazone. These data also suggest annual bluegrass cross-resistance with amicarbazone is a legitimate concern for turfgrass practitioners who are combating resistance to other triazine herbicides.

Sources of Materials

[1] Fafard potting mix, Conrad Fafard Inc., P.O. Box 790, Agawam, MA 01001.

[2] Miracle Grow Plant Food®, The Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, Ohio 43041.

[3] Amicarbazone® 70 DF herbicide, Arysta LifeScience Corp., 15401 Weston Parkway Suite 150, Cary, NC 27513.

[4] Aatrex® 4L herbicide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[5] Princep® 4L herbicide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[6] Induce®, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[7] Tee Jet® Spraying Systems Co. Wheaton, IL 60189-7900.

[8] OS1-FL® Modulated chlorophyll fluorometer, Opti-Sciences, Inc., 8 Winn Ave., Hudson, NH 03051.

[9] SAS® version 9.1, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

Literature Cited

Ali, A. and V. S. Machado. 1981. Rapid detection of 'triazine resistant' weeds using chlorophyll fluorescence. Weed Res. 21:191-197.

Brecke, B., D. O. Stephenson, and J. B. Unruh. 2005. Annual bluegrass control in overseeded bermudagrass. ASA-CSSA-SSSA International Meeting. No. 64-9.

Conard, S. G. and S. R. Radosevich. 1979. Ecological fitness of *Senecio vulgaris* and *Amaranthus retroflexus* biotypes susceptible or resistant to atrazine. J. of Appl. Ecol. 16:171-177.

Darmency, H. and J. Gasquez. 1983. Interpreting the evolution of a triazine-resistant population of *Poa annua* L. New Phytol. 95:229-304.

Dayan, F. E., M. L. B. Trindade, and E. V. Velini. 2009. Amicarbazone, a new photosystem II inhibitor. Weed Sci. 57:579-583.

Frans, R., R. Talbert, D. Marx, and H. Crowley. 1986. Experimental design and techniques for measuring and analyzing plant response to weed control practices. p. 37–38. *In* N. D. Camper (ed.), Research Methods in Weed Science, 3rd ed., Southern Weed Sci. Soc., Champaign, IL.

Genty, B., J-M. Briantais, and N.R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta. 990:87-92.

Gressel, J. 2002. Molecular Biology of Weed Control. Taylor and Francis. New York.

Habash, D., M. P. Percival, and N. R. Baker. 1985. Rapid chlorophyll fluorescence technique for the study of penetration of photosynthetically active herbicides into leaf tissue. Weed Res. 25:389-395.

Heap, I. 2011. International survey of herbicide resistant weeds. www.weedscience.com. Accessed: March 31, 2011.

Hirschberg, J. and L. McIntosh. 1983. Molecular basis of herbicide resistance in *Amaranthus hybridus*. Science. 222:1346-1349.

Holt, J. S. 1988. Reduced growth, competitiveness, and photosynthetic efficiency of triazine-resistant *Senecio vulgaris* from California. J. Appl. Ecol. 25:307-318.

Holt, J. S. and S. R. Radosevich. 1983. Differential growth of two common groundsel (*Senecio vulgaris*) biotypes. Weed Sci. 31:112-115.

Hutto, K. C., G. E. Coats, and J. M. Taylor. 2004. Annual bluegrass (*Poa annua*) resistance to simazine in Mississippi. Weed Technol. 18:846-849.

Kelly, S. T., G. E. Coats, and D. S. Luthe. 1999. Mode of resistance of triazine-resistant annual bluegrass (*Poa annua*). Weed Technol. 13:747-752.

Kumata, S., K. Yoneyama, M. Ogasawara, Y. Takeuchi, M. Konnai, Y. Nakajima, T. Nakano, and S. Yoshida. 2001. Molecular basis of resistance to s-triazine herbicides in *Poa annua* L. and its photosynthetic properties under different light conditions. J. Pesticide Sci. 26:236-243.

Littell, R. C, G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models 2nd ed. SAS Publishing. Cary, N.C.

Maxwell. K. and G. N. Johnson. 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Botany. 51:659-668.

McCullough, P. E., S. Hart, D. Weisenberger, and Z. Reicher. 2010. Amicarbazone efficacy on annual bluegrass and safety on cool season turfgrasses. Weed Technol. 24:461-470.

Menendez, J., J. Gonzalez-Gutierrez, and R. De Prado. 2006. Characterisation of a triazine-resistant biotype of *Bromus tectorum* found in Spain. Weed Res. 47:113-121.

Senseman, S. A. 2007. Herbicide Handbook 9th ed. Lawrence, Kansas. Weed Science Society of America. Pp. 128-129.

Shaw, D. R., T. F. Peeper, and D. L. Notziger. 1985. Comparison of chlorophyll fluorescence and fresh weight as herbicide bioassay techniques. Weed Sci. 33:29-33.

Toler, J. E., T. G. Willis, A. G. Estes, and L. B. McCarty. 2007. Postemergent annual bluegrass control in dormant nonoverseeded bermudagrass turf. HortSci. 42:670-672.

Walker, R. H. and J. L. Belcher. 2009. Annual bluegrass control with amicarbazone in perennial ryegrass overseed. Proc. South. Weed Sci. Soc. 62:191.

Yelverton, F. 2008. Spring transition from perennial ryegrass to bermudagrass and *Poa annua* management. Online. http://www.turffiles.ncsu.edu/PDFFiles/004633/Southern_California_Golf_Course_Superintendents_Association_Manhattan_Beach_CA.pdf.

Yelverton, F. and J. Isgrigg. 1998. Herbicide-resistant weeds in turfgrass. Golf Course Manage. 66:56-61.

Table 16. Annual bluegrass control following amicarbazone and triazine applications to triazine-resistant and -susceptible populations.

Herbicide	Population	1 WAT[a]	2 WAT
		% control[b]	
Amicarbazone (0.26 kg ai ha^{-1})	COM-01 (susceptible)	61	96
	AL-01 (susceptible)	89	100
	MS-01 (resistant)	0	0
	MS-02 (resistant)	0	0
Atrazine (1.7 kg ai ha^{-1})	COM-01 (susceptible)	82	98
	AL-01 (susceptible)	78	100
	MS-01 (resistant)	0	0
	MS-02 (resistant)	0	0
Simazine (1.7 kg ai ha^{-1})	COM-01 (susceptible)	36	95
	AL-01 (susceptible)	24	99
	MS-01 (resistant)	0	0
	MS-02 (resistant)	0	0
Nontreated		0	0
LSD (0.05)		10.3	3.98

[a] Abbreviation: WAT, Weeks After Treatment.

[b] Control data were similar across studies, therefore data were pooled.

Figure 8. Relative quantum yield of triazine-resistant and –susceptible annual bluegrass populations following treatment with amicarbazone, atrazine, and simazine.

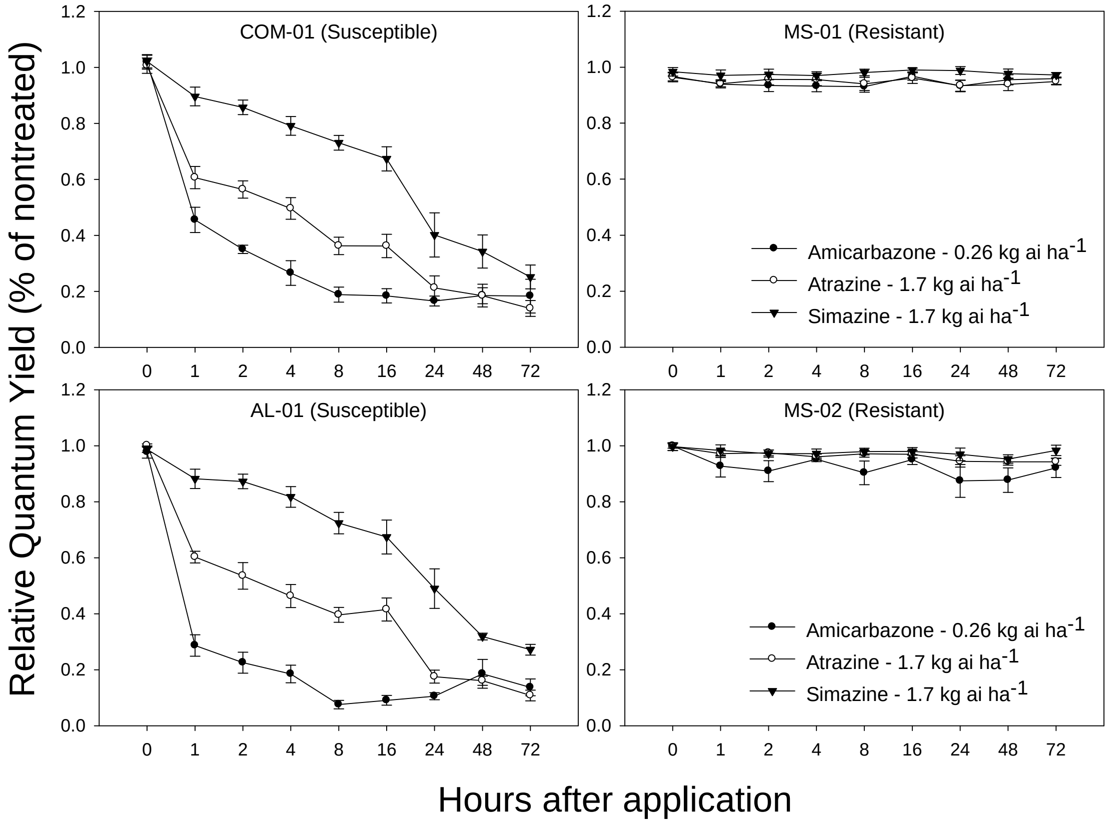
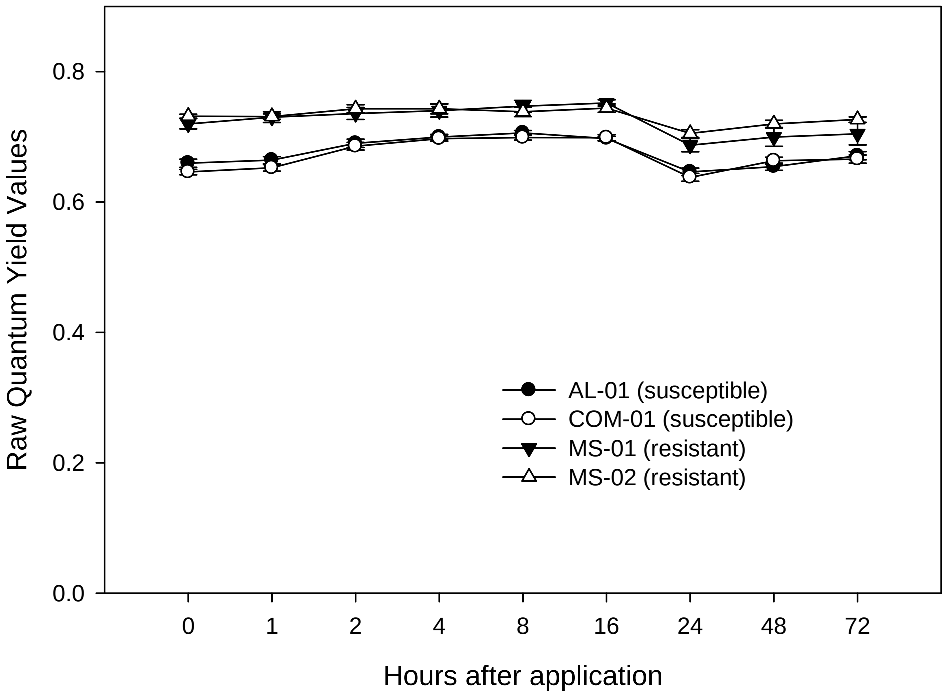


Figure 9. Raw quantum yield values of non-treated triazine-resistant and –susceptible annual bluegrass populations.



VI. Evaluation of Amicarbazone for Annual Bluegrass Control in Overseeded Perennial Ryegrass

Abstract

Amicarbazone reportedly controls annual bluegrass and its use in common bermudagrass overseeded with perennial ryegrass would be beneficial for turfgrass managers. Field studies were conducted to determine amicarbazone efficacy and safety in overseeding situations. One study evaluated amicarbazone tank-mix applications with ethofumesate. Treatments included amicarbazone applied alone at 0.065, 0.13, and 0.26 kg ai ha^{-1} or tank-mixed with ethofumesate at 1.13 kg ai ha^{-1}, ethofumesate applied at 1.13 followed by (fb) 1.13 kg ai ha^{-1}, and amicarbazone applied at 0.26 + ethofumesate applied at 0.57 kg ai ha^{-1}. Amicarbazone applied alone resulted in unacceptable annual bluegrass control. Tank-mixing amicarbazone with ethofumesate controlled annual bluegrass similar to traditional ethofumesate application regimes. All treatments resulted in less than 20% injury 2 and 4 WAIT. The second study evaluated single and sequential amicarbazone applications beginning in December or March for annual bluegrass control and perennial ryegrass overseed response. Treatments included amicarbazone applied alone or sequentially at 0.13 or 0.26 kg ai ha^{-1} beginning in December or March, ethofumesate applied in December at 2.1 or 1.05 fb 1.05 kg ai ha^{-1}, amicarbazone applied at 0.53 kg ai ha^{-1} in March, and bispyribac-sodium

(bispyribac) applied once or sequentially in March at 0.07 kg ai ha^{-1}. Single or sequential amicarbazone applications at 0.13 or 0.26 kg ai ha^{-1} did not provide acceptable annual bluegrass control. Amicarbazone applied at 0.53 kg ai ha^{-1} in March resulted in the best combination of annual bluegrass control and perennial ryegrass safety. Ethofumesate and bispyribac applied sequentially controlled annual bluegrass > 90% in April. These data suggest that amicarbazone applied at 0.53 kg ai ha^{-1} in March controls annual bluegrass and is safe on perennial ryegrass overseed; however, these applications are inferior to ethofumesate and bispyribac applied sequentially at 0.07 kg ai ha^{-1}.

Introduction

Perennial ryegrass is a cool-season turfgrass species commonly utilized in overseeding bermudagrass to provide green color during dormancy. Overseeding involves seeding into actively growing warm-season turfgrass in late-summer to early-fall (Puhalla et al. 1999). Perennial ryegrass possesses a dark-green color, fine-texture, and provides a durable and uniform playing surface in an otherwise dormant turf (Horgan and Yelverton 2001; Puhalla et al. 1999).

Annual bluegrass (*Poa annua* L.) is a weed species which commonly disrupts turfgrass aesthetics. Annual bluegrass' weedy characteristics include: a lighter-green leaf color than other desirable turfgrass species and profuse seedhead production (Beard 1973). Ethofumesate has been utilized since the early 1980's for POST annual bluegrass control in overseeding scenarios. Bermudagrass dormancy is required when making ethofumesate applications, or spring bermudagrass transition may be interrupted (Johnson 1983). Dickens (1979) observed bermudagrass injury following fall applications, but did not observe bermudagrass transition issues the following spring. Ross (2001) reported annual bluegrass control, but unacceptable perennial ryegrass injury when applied three times at 2-week intervals. Yelverton and McCarty (2001) observed > 95% annual bluegrass control following ethofumesate applications 6 and 9 WAO. McElroy et al. (2011) achieved > 90% annual bluegrass control with ethofumesate applied sequentially 12 and 15 WAO. Ethofumesate success may be attributed to annual bluegrass' ability to absorb more herbicide than perennial ryegrass and creeping bentgrass (Kohler and Branham 2002). Based on previous research, ethofumesate applications to perennial ryegrass overseed should be limited to December/early January applications or unacceptable bermudagrass injury may result.

Bispyribac-sodium (bispyribac) is registered for POST annual bluegrass control in bermudagrass overseeded with perennial ryegrass (Anonymous 2008). McElroy et al. (2011) observed acceptable annual bluegrass control with single and sequential bispyribac applications, but sequential applications significantly reduced perennial ryegrass cover when applied 12 and 15 WAO. Temperature is reported to affect bispyribac activity on annual bluegrass (McCullough and Hart 2006). Optimum annual bluegrass control in conjunction with minimum perennial ryegrass injury is expected with sunny conditions and daytime maximum air temperatures between 21 and 27 C (Anonymous 2008). Previous research suggests bispyribac applications to perennial ryegrass overseed may be limited to February/March, or unacceptable perennial ryegrass injury may result.

Amicarbazone is a photosystem II (PSII) inhibiting herbicide which halts electron transport by binding to the Q_B binding niche on the D1 protein of susceptible plants

(Dayan et al. 2009; Senseman 2007). Amicarbazone is being evaluated for selective removal of annual bluegrass from perennial ryegrass overseed (Walker and Belcher 2009). Walker and Belcher (2009) reported near complete control of annual bluegrass with March applications. Control was achieved with single or sequential amicarbazone applications with no adverse effects on spring bermudagrass transition (Walker and Belcher 2009). Spring amicarbazone applications reportedly provide the best combination of creeping bentgrass safety and annual bluegrass control (McCullough et al. 2010).

Research is needed to better understand amicarbazone use strategies in perennial ryegrass overseed. The objectives of these field studies were 1) to evaluate perennial ryegrass response and annual bluegrass control following amicarbazone and ethofumesate tank-mix applications and 2) to evaluate amicarbazone application timing versus industry standards on perennial ryegrass response and annual bluegrass control.

Materials and Methods

Description of Study Sites. Six separate studies over a 2-year period were conducted to evaluate annual bluegrass control and perennial ryegrass overseed response to herbicide amicarbazone and industry standard herbicides. Study sites included the Auburn University Turfgrass Research Unit (TGRU) in Auburn, AL and the Auburn University Plant Breeding Unit (PBU) in Tallassee, AL. The soil at the TGRU location was a Marvyn loamy sand (Marvyn fine-loamy, kaolinitic, thermic typic Kanhapludults) (pH = 6.1). The soil at the PBU location was a Wickham sandy loam (Wickham fine-loamy, mixed, semiactive, thermic typic hapludults) (pH = 5.6). The TGRU location was an existing stand of ‘Tifway’ hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. x *C.*

transvaalensis Burtt Davy] and the PBU location was common bermudagrass [*C. dactylon* (L.) Pers.]. ‘Goalkeeper’ perennial ryegrass was seeded on October 15, 2009 at 896 and 672 kg ha^{-1} at TGRU and PBU, respectively. ‘Replay ‘ perennial ryegrass was seeded at TGRU on October 13, 2010 at 896 kg ha^{-1}.

Amicarbazone/Ethofumesate Tank-Mix Study. Two field studies were conducted in 2009-2010 at TGRU and PBU, and one field study was initiated in 2010-2011 at the TGRU location. Herbicide treatments (Table 17) included amicarbazone[1] applied at 0.065, 0.13, or 0.26 kg ai ha^{-1} alone or in combination with ethofumesate[2] at 1.13 kg ai ha^{-1}, amicarbazone at 0.26 + ethofumesate at 0.57 kg ha^{-1}, and ethofumesate applied in single and sequential applications at 1.13 kg ha^{-1} served as standard treatments. A nonionic surfactant[3] was added to all treatments containing amicarbazone. Herbicide solutions were applied with a CO_2 pressurized backpack sprayer using 8002VS nozzles[4] calibrated to deliver 280 L ha^{-1} of spray solution at 138 kPa. Non-treated plots were included in all studies. Herbicide treatments were initiated on December 4 and December 16 for the 2009 and 2010 studies, respectively. The growth stage of perennial ryegrass at the time of initial application was 7 to 8 leaves. Experimental plots were arranged in randomized complete blocks with four replications each. Experimental units were 4.5 m^2 in 2009-2010 and 3.3 m^2 in 2010-2011. Plots were mown weekly at 2.5 cm and irrigated as needed.

Data collected included perennial ryegrass injury, cover, quality, color, and annual bluegrass control. Annual bluegrass control was rated on a percentage scale (0-100), where 0 equaled no control and 100 equaled complete control. Perennial ryegrass injury was visually rated on a percentage scale, where 0 equaled no chlorosis or necrosis and 100 equaled complete death of perennial ryegrass (Frans et al. 1986). For discussion purposes, 20% injury is the maximum level of injury considered to be acceptable by commercial standards (Johnson 1995; Johnson 1996; Johnson and Murphy 1995). Perennial ryegrass cover was rated on a percent scale where 0 equaled no perennial ryegrass and 100 equaled no reduction in cover. Perennial ryegrass quality was rated on a 0-9 scale where 0 equaled poor quality and 9 equaled optimum quality (Toubakaris and McCarty 2000). Perennial ryegrass color was measured utilizing a normalized difference vegetative index (NDVI) turf color meter[5]. Three subsample measurements were randomly taken from each plot. Using PROC SQL in SAS®[6], NDVI subsample measurements were averaged for each plot and then converted to a relative percentage of the non-treated plots within each trial, block, and rating date. Experimental data were subjected to analysis of variance ($P = 0.05$) using type III tests of fixed effects in PROC MIXED of SAS. Data from non-treated plots were removed during data analysis to improve homogeneity of variance. Pairwise contrasts were utilized for making preplanned comparisons for NDVI and annual bluegrass control according to the original experimental objectives (Bowley 2008). Experimental data for perennial ryegrass injury, quality, and NDVI were similar ($P > 0.05$) for the 2009 and 2010 studies. Therefore, data were pooled across experiments. Annual bluegrass control was similar across both locations in 2009, but different from the 2010 study. Therefore, both years are presented separately.

Amicarbazone vs. Industry Standards. Two field studies were conducted in 2009-2010 at TGRU and PBU, and one field study was initiated in 2010-2011 at the TGRU location. Herbicides (Table 18) were applied in December/January or March. Amicarbazone was applied at 0.13 or 0.26 kg ai ha^{-1} in single or sequential applications in December/January or March. Ethofumesate was applied at 2.1 or 1.05 followed by (fb) 1.05 kg ai ha^{-1} in the December/January, and bispyribac[7] was applied at 0.07 kg ai ha^{-1} in single and two sequential applications in March. A nonionic surfactant was added to herbicide solutions at 0.25% v/v. Sprayer calibration, application dates, plot size, design, and maintenance was the same as in the previously described studies.

Data collection and description were the same as in the previously described study; however, perennial ryegrass color and quality were not collected for this study. Experimental data were subjected to analysis of variance ($P = 0.05$) using type III tests of fixed effects in PROC MIXED of SAS®. Data from non-treated plots were removed during data analysis to improve homogeneity of variance. Pairwise contrasts were utilized for making preplanned comparisons for annual bluegrass control, perennial ryegrass injury and quality according to the original experimental objectives. Experimental data for perennial ryegrass injury and cover were similar ($P > 0.05$) for the 2009-2010 and 2010-2011 studies, so pooling of data was deemed appropriate. Annual bluegrass control was similar across both locations in 2009-2010, but different from the 2010-2011 study. Therefore, annual bluegrass control data for both years are presented separately.

Results and Discussion

Amicarbazone/Ethofumesate Tank-Mix Study. Herbicide treatment significantly affected ($P < 0.001$) perennial ryegrass injury 2 and 4 WAIT. Perennial ryegrass injury was first observed as a minor chlorosis of treated plots. Leaf-tip necrosis was observed on some perennial ryegrass plants. All herbicide treatments resulted in an acceptable level of injury 2 and 4 WAIT. Amicarbazone applied at 0.26 kg ha^{-1} alone or in combination with ethofumesate injured perennial ryegrass < 20% 2 WAIT (Table 19). Amicarbazone applied at 0.065 and 0.13 kg ha^{-1} alone or in combination with ethofumesate injured perennial ryegrass ≤10% 2 WAIT. Ethofumesate applied sequentially at 1.13 kg ha^{-1} injured perennial ryegrass 10% 4 WAIT. Injury increased when ethofumesate was tank-mixed with amicarbazone at 0.26 kg ha^{-1}. Minimal injury was observed on treated plots 6 WAIT.

Herbicide treatment significantly affected perennial ryegrass quality 2 ($p < 0.001$) and 4 ($P = 0.007$) WAIT. Amicarbazone applied at 0.13 and 0.26 kg ha^{-1} significantly reduced perennial ryegrass quality 2 WAIT (Table 19). Perennial ryegrass quality was exacerbated by ethofumesate tank-mixed with amicarbazone at 0.13 and 0.26 kg ha^{-1} 2 WAIT. Amicarbazone applied at 0.26 kg ha^{-1} + ethofumesate applied at 1.13 kg ha^{-1} reduced perennial ryegrass quality greater than other treatments 4 WAIT. Amicarbazone applied at 0.13 and 0.26 kg ha^{-1} tank-mixed with ethofumesate significantly reduced perennial ryegrass quality 4 WAIT. Ethofumesate applied sequentially at 1.13 kg ha^{-1} did not significantly reduce perennial ryegrass quality. These results differed from previous research which observed reduction in perennial ryegrass quality with ethofumesate (Johnson 1983; Rossi 2001). Bermudagrass spring transition was not adversely affected by any herbicide treatment.

Perennial ryegrass color was significantly affected by treatment ($P = 0.001$) 9 WAIT. All amicarbazone treatments resulted in increased perennial ryegrass color 9 WAIT (Table 20). Ethofumesate applied sequentially at 1.13 kg ha^{-1} did not affect perennial ryegrass color. Perennial ryegrass treated with ethofumesate alone had significantly less green color than perennial ryegrass treated with amicarbazone alone (P = 0.006). Perennial ryegrass treated with amicarbazone alone had similar color to amicarbazone tank-mixed with ethofumesate. Amicarbazone applied at 0.26 kg ha^{-1} tank-mixed with ethofumesate at 1.13 kg ha^{-1} resulted in similar turf color to amicarbazone applied at 0.26 kg ha^{-1} tank-mixed with ethofumesate at 0.57 kg ha^{-1}.

Herbicide treatments significantly affected annual bluegrass control in 2009 (P < 0.001) and 2010 (P < 0.001). In 2009 and 2010, annual bluegrass control increased as amicarbazone rate increased; however, acceptable control was not achieved (Table 20). Amicarbazone applied alone controlled annual bluegrass <30% in 2009. In 2009, ethofumesate applied sequentially at 1.13 kg ha^{-1} controlled annual bluegrass greater than all ethofumesate + amicarbazone tank-mixes. Amicarbazone tank-mixed with ethofumesate controlled annual bluegrass greater than amicarbazone applied alone at the same rate in 2009 and 2010. Treatment contrasts indicate ethofumesate at 0.57 kg ha^{-1} + amicarbazone at 0.26 kg ha^{-1} controlled annual bluegrass similar to ethofumesate at 1.13 kg ha^{-1}. In 2010, all amicarbazone + ethofumesate tank-mix applications resulted in near complete annual bluegrass control.

Amicarbazone vs. Industry Standards. Amicarbazone applied in single and sequential applications at 0.13 and 0.26 kg ha^{-1} controlled annual bluegrass the greatest when applied in the spring in 2010 (P = 0.031); however, in 2011 no significance was observed (P = 0.118) (Table 21). Amicarbazone applied at 0.13 and 0.26 did not provide acceptable annual bluegrass control in 2010 or 2011. Amicarbazone applied at 0.13 kg ha^{-1} in single and sequential applications in the fall controlled annual bluegrass 6 and 16%, respectively, in 2010. The same rate applied in single and sequential applications in the spring controlled annual bluegrass 7 and 8%, respectively, in 2010. Amicarbazone applied in fall controlled annual bluegrass greater in 2011, but control was less than 40%. Amicarbazone applied sequentially at 0.26 kg ha^{-1} controlled annual bluegrass 21 and 66% in 2010 and 2011, respectively. Amicarbazone applied at 0.53 kg ha^{-1} in March controlled annual bluegrass 74 and 75% in 2010 and 2011, respectively. Ethofumesate applied at 2.1 and 1.05 fb 1.05 kg ha^{-1} controlled annual bluegrass between 89 and 100% for all studies which was significantly better ($P < 0.001$) than amicarbazone. Bispyribac applied once at 0.07 kg ha^{-1} controlled annual bluegrass <15% in 2010 and 2011. Amicarbazone applied in the spring controlled annual bluegrass greater than single applications of bispyribac, but control was ≤75%. Bispyribac applied sequentially at 0.07 kg ha^{-1} controlled annual bluegrass >90% in 2010 and 2011. Similar annual bluegrass control with sequential applications of ethofumesate and bispyribac has been observed in previous research (McElroy et al. 2011; Dickens 1979; Johnson 1983).

Amicarbazone applied in the fall injured perennial ryegrass greater than spring applications ($P < 0.001$) (Table 21). Amicarbazone applied sequentially in the fall injured perennial ryegrass 19 and 40% at 0.13 and 0.26 kg ha^{-1}, respectively, 4 WAIT. These treatments led to reductions in cover of 12 and 20%, respectively. Amicarbazone applied at 0.13 and 0.26 kg ha^{-1} in the spring did not injure perennial ryegrass 4 WAIT. Amicarbazone applied at 0.53 kg ha^{-1} injured perennial ryegrass 5%. Ethofumesate injured perennial ryegrass less than 5% regardless of rate. Bispyribac applied sequentially at 0.07 kg ha^{-1} injured perennial ryegrass significantly 3 WAIT (data not shown); however, injury was 9% 4 WAIT. Amicarbazone applied in the spring provided the best combination of annual bluegrass control and perennial ryegrass safety suggesting mature perennial ryegrass was more tolerant than non-tillered plants. Amicarbazone use is cautioned prior to mid-spring or until winter stress has subsided (Anonymous 2011). Although amicarbazone significantly injured perennial ryegrass in the fall, perennial ryegrass maturation through tillering led to near complete cover in the spring. Amicarbazone may initially control seedling annual bluegrass in the fall; however, spring germination of annual bluegrass may be the culprit of poor control ratings in the spring (Kaminski and Dernoeden 2007). Annual bluegrass control in perennial ryegrass overseed is further complicated without a well-defined germination period (McElroy et al. 2011). Amicarbazone applied at 0.53 kg ha^{-1} in the spring did provide marginal annual bluegrass control with minimal perennial ryegrass injury, suggesting sequential applications at this rate may increase control levels. However, the projected maximum amicarbazone annual use rate is 0.50 kg ha^{-1} and multiple applications this late in the spring may not be warranted (Anonymous 2011).

Implications for Control. Amicarbazone does possess the capability of selectively controlling annual bluegrass in perennial ryegrass overseed. Amicarbazone applied at 0.26 kg ha^{-1} tank-mixed with ethofumesate controlled annual bluegrass similar to sequential ethofumesate applications, suggesting a sequential ethofumesate application could be eliminated. Sequential amicarbazone applications may be necessary to achieve acceptable annual bluegrass control, but these applications may be limited to mature perennial ryegrass in March and may require rates >0.26 kg ha^{-1}. Amicarbazone applied at 0.53 kg ha^{-1} provided the best combination of annual bluegrass control and perennial ryegrass safety; however, greater control was achieved with ethofumesate and bispyribac. More amicarbazone studies are needed to determine best annual bluegrass control strategies. Further research involving fall + spring sequential applications or pre-overseeding amicarbazone applications may provide a different approach to controlling annual bluegrass in bermudagrass overseeded with perennial ryegrass.

Sources of Materials

[1] Amicarbazone® 70 DF herbicide, Arysta LifeScience Corp., 15401 Weston Parkway Suite 150, Cary, NC 27513.

[2] Prograss® herbicide, Bayer Environmental Science, 95 Chestnut Ridge Rd., Montvale, NJ 07645.

[3] Induce®, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[4] Tee Jet® Spraying Systems Co. Wheaton, IL 60189-7900.

[5] Field Scout TCM 500 NDVI Turf Color Meter, Spectrum Technologies, Inc., 12360 S. Industrial Dr. East, Plainfield, IL 60585.

[6] SAS® version 9.1, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

[7] Velocity® Herbicide, Valent Professional Products, P. O. Box 8025, Walnut Creek, CA 94596.

Literature Cited

Anonymous. 2011. Xecute 70WDG Technical bulletin. Arysta LifeScience Corp., 15401 Weston Parkway Suite 150, Cary, NC 27513.

Anonymous. 2008. Velocity SG Herbicide. Valent U.S.A. Corporation. Walnut Creek, CA 94596.

Beard, J. B. 1973. Turfgrass: Science and Culture. Prentice Hall. New Jersey. Pp. 67-68.

Bowley, S. R. 2008. A Hitchhiker's Guide to Statistics in Plant Biology. 2nd ed. Any Old Subject Books. Ontario. Pp. 42-44.

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

Dickens, R. 1979. Control of annual bluegrass (*Poa annua*) in overseeded bermudagrass (*Cynodon* spp.) golf greens. Weed Sci. 27:642-644.

Frans, R., R. Talbert, D. Marx, and H. Crowley. 1986. Experimental design and techniques for measuring and analyzing plant response to weed control practices. p. 37–38. *In* N. D. Camper (ed.), Research Methods in Weed Science, 3rd ed., Southern Weed Sci. Soc., Champaign, IL.

Horgan, B. P. and F. H. Yelverton. 2001. Removal of perennial ryegrass from overseeded bermudagrass using cultural methods. Crop Sci. 41:118–126.

Johnson, B. J. 1983. Response to ethofumesate of annual bluegrass (*Poa annua*) and overseeded bermudagrass (*Cynodon dactylon*). Weed Sci. 31:385-390.

Johnson, B. J. 1995. Tolerance of four seeded common bermudagrass (*Cynodon dactylon*) types to herbicides. Weed Technol. 9:794-800.

Johnson, B. J. 1996. Tank-mixed postemergence herbicides for large crabgrass (*Digitaria sanguinalis*) and goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) turf. Weed Technol. 10:716-721.

Johnson, B. J. and T. R. Murphy. 1995. Effect of paclobutrazol and flurprimidol on suppression of Poa annua spp. reptans in creeping bentgrass (Agrostis stolonifera) greens. Weed Technol. 9:182-186.

Kaminski, J. E. and D. H. Dernoeden. 2007. Seasonal *Poa annua* L. seedling emergence patterns in Maryland. Crop Sci. 47:775-781.

Kohler, E. A. and B. E. Branham. 2002. Site of uptake, absorption, translocation, and metabolism of ethofumesate in three turfgrass species. Weed Sci. 50:576-580.

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.

McCullough, P. E., and S. E. Hart. 2006. Temperature influences creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*) response to bispyribac-sodium. Weed Technol. 20:728-732.

McElroy, J. S., G. K. Breeden, and G. Wehtje. 2011. Evaluation of annual bluegrass control programs for bermudagrass turf overseeded with perennial ryegrass. Weed Technol. 25:58-63.

Puhalla, J., J. Krans, and M. Goatley. 1999. Sports Fields: A Manual For Design Construction and Maintenance. John Wiley & Sons, Inc. New Jersey. Pp. 24-26, 269.

Rossi, F. S. 2001. Annual bluegrass population dynamics in response to growth regulators and herbicides. Int. Turfgrass Soc. Res. J. 9:906-909.

Senseman, S. A. 2007. Herbicide Handbook. Lawrence, KS: Weed Science Society of America. 11 p.

Toubakaris, M. and B. McCarty. 2000. Heat stress separates old and new bentgrasses: Cultivars vary in their responses to summertime stress. Golf Course Manage. 68:49-53.

Walker, R. H. and J. L. Belcher. 2009. Annual bluegrass control with amicarbazone in perennial ryegrass overseed. Proc. of South. Weed Sci. Soc. 62:191.

Yelverton, F. H. and L. B. McCarty. 2001. Tolerance of perennial ryegrass and *Poa annua* control with herbicides in overseeded bermudagrass. Int. Turfgrass Soc. Res. J. 9:1050-1055.

Table 17. Treatment structure of field studies initiated in 2009 and 2010.

Treatment[a]	Rate kg ai ha^{-1}	Timing[b] WAO[c]
Amicarbazone	0.065	7
Amicarbazone	0.13	7
Amicarbazone	0.26	7
Ethofumesate	1.13	7
Ethofumesate fb ethofumesate	1.13 fb 1.13	7 fb 10
Amicarbazone + ethofumesate	0.065 + 1.13	7
Amicarbazone + ethofumesate	0.13 + 1.13	7
Amicarbazone + ethofumesate	0.26 + 1.13	7
Amicarbazone + ethofumesate	0.26 + 0.57	7

[a] Amicarbazone = Amicarbazone® 70 DF herbicide, ethofumesate = Prograss® Herbicide.

[b] Studies were initiated on December 4, 2009 and December 11, 2010 with the sequential application on December 26, 2010 and January 3, 2011.

[c] Abbreviations: fb = followed by; WAO = Weeks After Overseeding.

Table 18. Herbicide rates, timings, and perennial ryegrass growth stage for field studies evaluating perennial ryegrass response and annual bluegrass control.

			Timing		
Treatment	Herbicide[a]	Rate (kg ai ha^{-1})	2009-2010[b]	2010-2011	Perennial ryegrass growth stage
	Fall				
1	Amicarbazone	0.13	December 4	December 16	≈ 7 leaf
2	Amicarbazone	0.13 fb[c] 0.13	December 4, 26	December 16, January 6	≈ 7 leaf
3	Amicarbazone	0.26	December 4	December 16	≈ 7 leaf
4	Amicarbazone	0.26 fb 0.26	December 4, 26	December 16, January 6	≈ 7 leaf
5	Ethofumesate	2.1	December 4	December 16	≈ 7 leaf
6	Ethofumesate	1.05 fb 1.05	December 4, 26	December 16, January 6	≈ 7 leaf
	Spring				
7	Amicarbazone	0.13	March 4	March 2	> 5 tiller
8	Amicarbazone	0.13 fb 0.13	March 4, 26	March 2, 23	> 5 tiller
9	Amicarbazone	0.26	March 4	March 2	> 5 tiller
10	Amicarbazone	0.26 fb 0.26	March 4, 26	March 2, 23	> 5 tiller
11	Amicarbazone	0.53	March 4	March 2	> 5 tiller
12	Bispyribac	0.074	March 4	March 2	> 5 tiller
13	Bispyribac	0.074 fb 0.074	March 4, 26	March 2, 23	> 5 tiller

[a] Amicarbazone = Amicarbazone® 70DF; ethofumesate = Prograss® Herbicide; bispyribac = Velocity® 80SP.

[b] Two studies were conducted at two locations in 2009-2010.

[c] Abbreviations: fb = followed by.

Table 19. Perennial ryegrass injury and quality following amicarbazone and ethofumesate applications, 2009-2010.

Treatment[a]	Rate kg ai ha^{-1}	2 WAIT[b]	4 WAIT	2 WAIT	4 WAIT
		% injury[c]		quality[d]	
Amicarbazone	0.065	1	6	5.8	6.3
Amicarbazone	0.13	7	10	5.5	5.7
Amicarbazone	0.26	17	18	5.4	5.8
Ethofumesate fb ethofumesate	1.13 fb 1.13	3	10	5.7	5.9
Amicarbazone + ethofumesate	0.065 + 1.13	5	9	5.6	6.0
Amicarbazone + ethofumesate	0.13 + 1.13	10	9	5.1	5.7
Amicarbazone + ethofumesate	0.26 + 1.13	19	17	5.2	5.4
Amicarbazone + ethofumesate	0.26 + 0.57	16	17	5.2	5.7
Nontreated	--	0	0	6.0	6.2
LSD ($\alpha = 0.05$)		4.4	3.1	0.4	0.5

[a] Amicarbazone = Amicarbazone® 70 DF herbicide; ethofumesate = Prograss® Herbicide.

[b] Abbreviations: WAIT = Weeks After Initial Treatment; fb = followed by; LSD = least significant difference.

[c] Perennial ryegrass injury was rated on a percent scale (0-100) where 0 = no injury and 100 = complete death of ryegrass.

[d] Perennial ryegrass quality was rated on a 0-9 scale where 0 equaled poor quality and 9 equaled optimum quality.

Table 20. Perennial ryegrass color[a] and annual bluegrass control following amicarbazone and ethofumesate applications, 2009-2010.

		Perennial ryegrass NDVI	Annual bluegrass control[b]	
	Rate		2009	2010
Treatment[c]	kg ai ha^{-1}	9 WAIT[d]	16 WAIT	15 WAIT
		% of nontreated	———% control———	
Amicarbazone	0.065	102.0	4	6
Amicarbazone	0.13	101.2	16	18
Amicarbazone	0.26	102.0	29	38
Ethofumesate fb ethofumesate	1.13 fb 1.13	100.0	96	99
Amicarbazone + ethofumesate	0.065 + 1.13	100.4	85	98
Amicarbazone + ethofumesate	0.13 + 1.13	101.0	89	100
Amicarbazone + ethofumesate	0.26 + 1.13	102.3	91	100
Amicarbazone + ethofumesate	0.26 + 0.57	102.0	88	100
Nontreated	--	--	0	0
LSD ($\alpha = 0.05$)		1.4	5	5
Pairwise contrast				
Amicarbazone alone vs. ethofumesate alone		0.0060	<0.0001	<0.0001
Amicarbazone alone vs. amicarbazone vs. ethofumesate				
Ethofumesate at 0.57 kg ha^{-1}(tank-mixed with amicarbazone at 0.26 kg ha^{-1}) vs. ethofumesate at 1.13 fb 1.13 kg ha^{-1}		0.3820	<0.0001	<0.0001
		0.6920	0.1840	1.000

[a] Perennial ryegrass color was determined using an NDVI turf color meter, and resulting values were calculated as relative values compared to the non-treated.

[b] Annual bluegrass control was pooled for the 2009 studies, and the 2010 study is presented separately.

[c] Amicarbazone = Amicarbazone® 70 DF; ethofumesate = Prograss® Herbicide.

[d] Abbreviations: WAIT = Weeks After Initial Treatment, fb = followed by, LSD = least significant difference.

[e] Contrasts with values less than 0.05 are significant.

Table 21. Annual bluegrass control and perennial ryegrass response following herbicide treatments.

			Annual bluegrass[a]		Perennial ryegrass	
Herbicide	Rate (kg ai ha^{-1})		2010	2011	4 WAIT[b]	4 WAIT
Fall			——% control——		% injury	% reduction[c]
Amicarbazone	0.13		6	4	5	5
Amicarbazone	0.13 fb 0.13		16	28	19	12
Amicarbazone	0.26		12	32	10	10
Amicarbazone	0.26 fb 0.26		31	39	40	20
Ethofumesate	2.1		95	89	3	3
Ethofumesate	1.05 fb 1.05		100	96	4	1
Spring						
Amicarbazone	0.13		7	3	0	0
Amicarbazone	0.13 fb 0.13		8	23	0	0
Amicarbazone	0.26		15	20	0	0
Amicarbazone	0.26 fb 0.26		21	66	0	0
Amicarbazone	0.53		74	75	5	0
Bispyribac	0.07		13	10	0	0
Bispyribac	0.07 fb 0.07		92	94	9	0
Nontreated	--		0	0	0	-
Pairwise contrast[d]		Treatment combinations				
Amicarbazone applied at 0.13 and 0.26 kg ha^{-1} (fall vs. spring)		1,2,3,4 vs. 7,8,9,10	0.0314	0.1183	<0.0001	<0.0001
Amicarbazone (0.13 vs. 0.13 fb 0.13 kg ha^{-1})		1,7 vs.2,8	0.0131	<0.0001	0.0002	0.0779
Amicarbazone (0.26 vs. 0.26 fb 0.26 kg ha^{-1})		3,9 vs. 4,10	<0.0001	<0.0001	<0.0001	0.0736
Amicarbazone (0.53 in spring vs. 0.26 kg ha^{-1} sequential)		11 vs. 10	<0.0001	<0.0001	<0.0001	<0.0001
Ethofumesate sequential vs. amicarbazone sequential		6 vs. 2,4,8,10	<0.0001	<0.0001	<0.0001	<0.0001
Bispyribac sequential vs. amicarbazone single in spring		13 vs. 8,10	0.0002	<0.0001	<0.0001	1.000

[a] Annual bluegrass control was rated on April 8 and April 18 in 2010 and 2011, respectively.

[b] Abbreviations: WAIT = Weeks After Initial Treatment (for fall and spring applications), fb = followed by.

[c] Perennial ryegrass cover was measured as a percent reduction of the non-treated 4 WAIT for each season.

[d] P-values less than 0.05 are statistically significant.