

EVALUATION OF AMICARBAZONE BEHAVIOR, SELECTIVITY, AND UTILIZATION
IN TURFGRASS

by

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(Under the Direction of Patrick E. McCullough)

ABSTRACT

Amicarbazone is a photosystem II inhibitor with potential for controlling annual bluegrass (*Poa annua* L.) in turfgrasses. Comprehensive investigations are required to evaluate application rates, regimens, timings, and mechanisms of selectivity to help maximize efficacy of this herbicide in turfgrass.

Amicarbazone selectivity for annual bluegrass control in cool-season turfgrasses could be attributed to differential levels of absorption, translocation, and metabolism. Annual bluegrass showed more absorption and translocation but less metabolism of amicarbazone compared to creeping bentgrass (*Agrostis stolonifera* L.) and tall fescue (*Festuca arundinaceae* Shreb.). Results of greenhouse experiments indicated that temperature has greater phytotoxic effect on turfgrass injury and shoot biomass reductions of annual bluegrass and tall fescue than bermudagrass following amicarbazone applications. Increased tall fescue injury from summer amicarbazone applications are likely attributed to effects of temperature on herbicide phytotoxicity. Foliar amicarbazone uptake in bermudagrass was comparable to tall fescue but less than annual bluegrass at low temperatures (25/20 °C). Foliar and root uptake in annual

bluegrass, bermudagrass, and tall fescue increased as temperature increased but uptake in bermudagrass was less than annual bluegrass and tall fescue at high temperatures (40/35 °C). Moreover, practitioners may safely use amicarbazone in seashore paspalum in winter, spring, and summer at labeled use rates but summer applications may inhibit shoot growth for up to 4 weeks.

INDEX WORDS: Amicarbazone, Annual bluegrass (*Poa annua* L.), Herbicide, Turfgrass

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DEDICATION

I would dedicate this dissertation to my wonderful family.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Annual Bluegrass

Annual bluegrass (*Poa annua* L.) is the most problematic weed in turfgrass. It is a member of the *Poaceae* family, *Pooideae* subfamily and has annual and perennial biotypes (GRIN 2014). The annual biotype of annual bluegrass has an erect growth habit and prolific seedhead production, but is highly variable in morphological characteristics (Beard et al. 1978; Hovin 1957; Youngner 1959). Annual bluegrass is a winter annual species that germinates in fall and completes its lifecycle in late spring. Perennial biotypes of annual bluegrass have stoloniferous growth that roots at the nodes with limited seed production (Beard et al. 1978; Hovin 1957; Warwick 1979). Annual and perennial biotypes of annual bluegrass do not represent a good subspecies because they are not geographically isolated and are present in various proportions in any given population (Warwick 1979).

Annual bluegrass (*Poa annua* L.) is proposed to have originated from Europe, but can be found throughout the world. It adapts to a wide range of climatic conditions including the subarctic, temperate, and subtropical areas of the world (Hovin 1957; Tutin 1957; Vargas and Turgeon 2004). Compared to most turf species, annual bluegrass has a lighter green color, coarser leaf texture, and produces unsightly seedheads that reduce turfgrass quality (Lush 1989). Additionally, annual bluegrass has poor tolerances to diseases including anthracnose (*Colletotrichum cereale*), dollar spot (*Rutstroemia floccosum*), necrotic ring spot

(*Leptosphaeria korrae*), pythium blight (*Pythium* spp.), yellow patch (*Rhizoctonia cerealis*), and summer patch (*Magnaporthe poae*) (Vargas and Turgeon 2004). The occurrence of these diseases may increase the use of fungicides for successful turfgrass culture.

Annual bluegrass is adapted to low mowing heights and is able to produce seedheads at 4 mm. Consequently, annual bluegrass is a common problematic weed species on closely mown turf sites such as golf course greens, tees, and fairways (Beard et al. 1978; Christians 2004; Toler et al. 2007). The optimum temperature range for annual bluegrass growth is 16 to 21 °C (Beard et al. 1978). Annual bluegrass decreases in summer due to poor heat and drought tolerances (Vargas and Turgeon 2004). Beard (1973) noted that the capability of annual bluegrass tolerance to temperature extremes is lower than creeping bentgrass (*Agrostis stolonifera* L.) and Kentucky bluegrass (*Poa pratensis* L.).

Soil fertility and pH level influence annual bluegrass populations in turfgrass (Lucas and Davis 1961; Varco and Sartain 1984). For example, in colonial bentgrass (*Agrostis capillaris* L.), urea applications at 586 kg N ha⁻¹ yr⁻¹ significantly increased annual bluegrass populations as compared to 293 and 976 kg N ha⁻¹ yr⁻¹ (Goss et al. 1975). In creeping bentgrass, annual bluegrass encroachment was enhanced with increased phosphorus and potassium fertilizer rates (Lawson 1999; Waddington 1978). Sartain (1985) noted that annual bluegrass infestation in bermudagrass (*Cynodon dactylon* (L.) Pers.) increased from 0 to 63% as soil pH increased from 5 to 5.8. Accordingly, sulfur applications have shown lowering soil pH to prevent annual bluegrass encroachment in turfgrasses (Goss et al. 1975; Varco and Sartain 1984).

Chemical Control of Annual Bluegrass in Turfgrass

Soil fumigants, plant growth regulators (PGRs), and herbicides are used for managing annual bluegrass in turfgrass polyculture (Teuton et al. 2007; Park and Landschoot 2003; Ohr et al. 1996). Non-selective soil fumigation has been described as the only approach to eradicate annual bluegrass populations from a turf site (Christians 1996). Soil fumigation with methyl bromide controls annual bluegrass. However, the registration of methyl bromide has been phased out in the United States in 2005 due to concerns over depletion of the ozone layer (Noling 1996; US Environmental Protection Agency 2014; Zhang et al. 2008). Methyl bromide alternatives such as methyl iodide and dazomet are available to turfgrass managers (Park and Landschoot 2003; Ohr et al. 1996; Zhang et al. 2008). Park and Landschoot (2003) reported dazomet at 388 kg ha⁻¹ with no plastic cover provided 92 to 97% annual bluegrass control. The researchers also noted creeping bentgrass establishment was not affected when seeded 3 d following the application of dazomet at 388 kg ha⁻¹.

PGRs interfere with plant hormone synthesis and regulate growth and development (Grossmann 1990; Phinney and West 1960; Rademacher 2000; Sorrell and Francis 2001). Early versions of PGRs were mainly used to reduce mowing frequency along roadsides and airport runways. The PGRs introduced during the 1980s and 1990s were generally safer, which allowed their uses in sports and lawn turfs (Vargas and Turgeon 2004). Type I PGRs such as mefluidide and maleic hydrazide inhibit cell division and differentiation in plant meristematic region. Some herbicides such as chlorsulfuron, glyphosate, imazameth, and sethoxydim also regulate plant growth when used at low rates (Fry and Huang 2004). Type II PGRs such as flurprimidol, paclobutrazol, and trinexapac-ethyl inhibit gibberellic acid production. Some fungicides such as

propiconazole, tradimefon, and fenarimol interfere with cytochrome P450 enzymes that may also retard turfgrass growth (Fry and Huang 2004).

Paclobutrazol and flurprimidol inhibit early gibberellin biosynthesis and suppress annual bluegrass greater than creeping bentgrass. This allows creeping bentgrass to have a competitive advantage in mixed stands for suppressing annual bluegrass (Graebe and Hedden 1985; Johnson and Murphy 1996; McCullough 2005; Rademacher 2000; Yelverton et al. 1999). Isgrigg et al. (1998) reported paclobutrazol more effectively inhibited annual bluegrass photosynthesis and tissue production than creeping bentgrass. They also noted annual bluegrass recovered about two weeks later than creeping bentgrass following the treatment of paclobutrazol in greenhouse. Isgrigg and Yelverton (1999) applied paclobutrazol or flurprimidol in spring or fall and reduced at least 40 and 80% annual bluegrass populations by the end of the first and second year, respectively. In other reports, Woosley et al. (2003) noted multiple paclobutrazol applications at 0.14 and 0.28 kg ha⁻¹ controlled greater than 85% annual bluegrass populations in creeping bentgrass fairways. McCullough et al. (2005) also reported similar level of annual bluegrass suppression by applying paclobutrazol in creeping bentgrass fairways. Unfortunately, the use of PGRs only suppresses annual bluegrass populations but do not provide complete control (Cooper et al. 1987; McCullough et al. 2005).

Preemergence (PRE) herbicides such as bensulide, dithiopyr, and prodiamine are commonly applied in cool-season turfgrasses for preventing annual bluegrass emergence (Bhowmik 1988; Bhowmik and Bingham 1990; Callahan and McDonald 1992; Stickler et al. 1969). However, most PRE herbicides lack herbicidal effect on established plants and will not effectively control perennial biotypes of annual bluegrass (Callahan and McDonald 1992). Efficacy of PRE herbicides depends on application timing, thatch thickness, application method,

soil moisture and temperature, and soil texture (Bhowmik 1988; Bhowmik and Bingham 1990; Stickler et al. 1969; Savage 1978). PRE herbicides should be applied before annual bluegrass emergence. However, fluctuation in environmental conditions may cause several peaks of annual bluegrass germination (McElroy et al. 2004) and PRE herbicides may offer inconsistent control (Kaminski and Dernoeden 2007). Reseeding needs to be delayed for several weeks or months following the application of some PRE herbicides due to their relative long soil residual activity (Bingham and Schmidt 1983; Johnson and Murphy 1991). Moreover, annual bluegrass populations resistant to dithiopyr, ethofumesate, prodiamine, and pendimethalin have been identified on golf courses in the United States (Heap 2014). Repeated use of these PRE herbicides may further increase the presence of herbicide resistant annual bluegrass populations (Isgrigg 2002; Gressel and Segel 1978; Lush 1989).

Currently, turf manager have limited POST herbicide options for selective control annual bluegrass in cool-season turfgrasses. Bispyribac-sodium selectively controls annual bluegrass with relative safety to creeping bentgrass, perennial ryegrass (*Lolium perenne* L.), and tall fescue (*Festuca arundinaceae* Shreb.) (Lycan and Hart 2006; McDonald et al. 2006; McCullough and Hart 2009a,b; Park et al. 2002). However, injury to other cool-season turfgrasses from bispyribac-sodium such as colonial bentgrass and Kentucky bluegrass is excessive (>20% injury) (Kaminski and Putman 2009; Lycan and Hart 2005). Inconsistent turf safety and annual bluegrass control from bispyribac-sodium is also attributed to seasonal application timing and temperature (Hart and McCullough 2007; Lycan and Hart 2006; McCullough and Hart 2006). Field experiments conducted by Lycan and Hart (2006) indicated that summer bispyribac-sodium applications were more effective than spring or fall applications for controlling annual bluegrass. They also noted that spring or autumn bispyribac-sodium applications may reduce

creeping bentgrass quality when temperatures are consistently less than 15 °C. These results agree with McCullough and Hart (2006) that noted annual bluegrass and creeping bentgrass exhibited differential responses after bispyribac-sodium application in which annual bluegrass was more sensitive to bispyribac-sodium at high temperature (20 and 30 °C) than low temperature (10 °C). In contrast, creeping bentgrass have greater sensitivity to bispyribac-sodium at low temperature (10 °C) than high temperature (20 and 30 °C).

Ethofumesate has efficacy for controlling annual bluegrass from PRE or postemergence (POST) applications (Hagggar and Passman 1981; Jukes and Goode 1981; Lee 1977; Lee 1981; Shearman 1986; Woosley et al. 2003). Jukes and Goodes (1981) noted ethofumesate at 5 and 10 kg ha⁻¹ causes annual bluegrass and perennial ryegrass cell division abnormalities. However, perennial ryegrass damage was insignificant and annual bluegrass damage was irreversible. Similarly, Coats and Krans (1986) reported perennial ryegrass had superior tolerance to ethofumesate compared to that of annual bluegrass. Lee (1981) noted annual bluegrass population was eliminated in perennial ryegrass seed fields after the applications of ethofumesate at 1.4 to 4.5 kg ha⁻¹. In other reports, ethofumesate provided erratic annual bluegrass control and inconsistent turf tolerance (Coat and Krans 1986; Dernoeden and Turner 1988; Shearman 1986). Coats and Krans (1986) noted ethofumesate at 1.1 kg ha⁻¹ did not significantly reduce ‘Adelphi’ Kentucky bluegrass stand counts, but other cultivars of cool-season turfgrasses including creeping bentgrass, colonial bentgrass, Kentucky, and rough bluegrass (*Poa trivialis* L.) were completely eliminated.

Mesotrione is a PRE herbicide but also provides POST control of annual bluegrass (Anonymous 2013; Askew et al. 2003; Hart and McCullough 2009). Newly seeded or established cool-season turfgrasses including Kentucky bluegrass, perennial ryegrass, and tall

fescue are tolerant to mesotrione (Askew et al. 2003; Blume 2009; S Bhowmik 2009; Hart and McCullough 2009; McElroy and Breeden 2007). Branham et al. (2010) reported that five treatments of mesotrione at 0.11 kg ha^{-1} applied twice per week provided greater than 95% annual bluegrass control in Kentucky bluegrass. Similarly, Skelton et al. (2012) found ten applications of mesotrione at 0.056 kg ha^{-1} with a frequency of two or three times per week or seven applications at 0.084 kg ha^{-1} with a frequency of twice per week provided consistent high level of annual bluegrass control. However, field experiments conducted by Reicher et al. (2011) in Indiana and Illinois noted an erratic annual bluegrass control from mesotrione applications across years and locations. Three mesotrione applications at 0.28 and 0.39 kg ha^{-1} at weekly intervals provided $>80\%$ annual bluegrass control in Indiana in 2005, whereas the same application regimens provided $<40\%$ control in Illinois in 2005 and 2006.

Overall, annual bluegrass is a major problematic weed in athletic fields, home lawns, and golf courses (Beard et al. 1978; Lush 1988; Lush 1989; Toler et al. 2007). Practitioners have limited effective herbicides to selectively control annual bluegrass in cool-season turfgrasses due to inconsistent efficacy and turfgrass safety (Coats and Krans 1986; McCullough et al. 2009a,b; Reicher et al. 2011; Teuton et al. 2007).

Annual Bluegrass Control in Seashore Paspalum

Seashore paspalum (*Paspalum vaginatum* Swartz) is a warm-season turfgrass used for golf greens, tees, fairways, and roughs (Dudeck and Peacock 1985; Duncan 1994; Duncan and Carrow 2000). Seashore paspalum exhibits good tolerance to adverse environmental stresses such as drought, water logging, wearing, and salt-laden soil (Brosnan and Deputy 2009; Duncan 1994; Dudeck and Peacock 1985; Trenholm et al. 1999; Trenholm et al. 2000; Marcum and

Murdoch 1994). Seashore paspalum is commonly planted on sites with high salinity that other turf species cannot tolerate (Emmons 2000). Some seashore paspalum cultivars with extraordinary salinity tolerance could be irrigated with ocean water (Duncan et al. 2000; Duncan and Carrow 2000). However, turf managers have limited POST herbicide options for selectively control annual bluegrass without injuring seashore paspalum (McCullough et al. 2012).

Various sulfonyleureas such as foramsulfuron, flazasulfuron, and trifloxysulfuron were reported to effectively control annual bluegrass but applications of these herbicides during spring green-up or actively growing seashore paspalum may cause unacceptable turf injury (Brosnan et al. 2010; McCullough et al. 2012; Wells et al. 2004; Wills et al. 2007). POST application of ethofumesate controls annual bluegrass (Coats and Krans 1986; Willis et al. 2006; Woosley et al. 2003) but rates required for annual bluegrass control cause excessive injury to seashore paspalum (Unruh et al. 2006). Field experiments conducted at Naples, Florida by Unruh et al. (2006) showed that POST ethofumesate application at 3400 g ha⁻¹ caused 30 and 60% 'Salam' seashore paspalum injury by 7 and 15 d after treatment (DAT) in 2000, while injury was less than 14% by 7 and 15 DAT in 2001.

Annual bluegrass could be controlled from PRE or POST pronamide applications (Anonymous 2009). McCullough et al. (2012) noted that pronamide is safe and effective for use on seashore paspalum during dormancy, spring green-up, or during active growth in spring. These researchers noted that seashore paspalum injury was minimal (5% or less) from pronamide at rates 0.84 to 3.36 kg ha⁻¹ by 4 week after treatment (WAT), while atrazine, bispyribac-sodium, and trifloxysulfuron caused excessive injury (>20%). Pronamide is absorbed in roots of established plants and translocated upward through apoplast (Carlson et al. 1975). Rainfall or overhead irrigation is essential after pronamide applications for obtaining optimum weed control.

However, excessive rainfall or irrigation (greater than 2.54 cm of water) may decrease the effectiveness of pronamide (Anonymous 2009). Pronamide phytotoxicity is inversely correlated with soil organic matter (Anonymous 2009; Connin et al. 1970; Carlson et al. 1975; Dutt and Harvery 1980) and it is a slow-acting herbicide for annual bluegrass control (Horgan and Yelverton 2001; McCullough et al. 2012; Yelverton 2004). Moreover, pronamide is prone to move laterally and treatments are not recommended on areas upslope of sensitive species (Anonymous 2009; Askew 2011; Senseman 2007).

Amicarbazone

Amicarbazone [4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] is a Photosystem II (PS II) inhibitor with similar activity to atrazine (Senseman 2007) (Figure 1). Due to lower use rates of amicarbazone, it was considered a more potent PS II inhibitor compared to atrazine (Dayan et al. 2009; Philbrook et al. 1999). Amicarbazone controls a broad spectrum of weeds including common lambsquarters (*Chenopodium album* L.), common cocklebur (*Xanthium strumarium* L.), and morningglory species (*Ipomoea* spp) in corn (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) (Philbrook et al. 1999; Senseman 2007; Seeruttun et al. 2008). Susceptible plants treated with amicarbazone exhibited chlorosis, stunted growth, tissue necrosis starting from leaf margin and progressing across entire leaf and stem, and eventual death (Dayan et al. 2009; Senseman 2007).

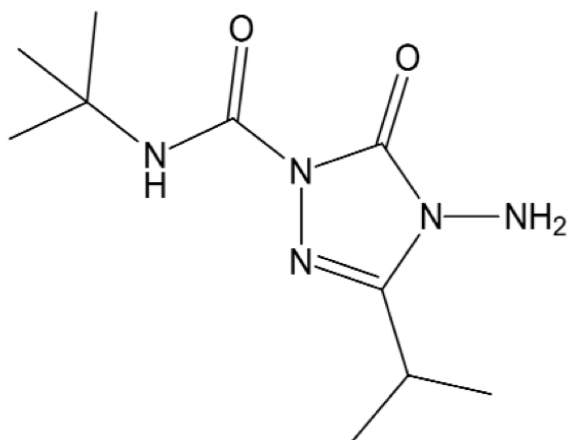


Figure 1.1. Amicarbazone chemical structure

In recent years, researchers noted amicarbazone has potential to selectively control annual bluegrass (*Poa annua* L.) in turfgrasses (Belcher and Walker 2009; Jeffries et al. 2013; Perry et al. 2011; McCullough 2010; Warren et al. 2009). Cool-season turfgrasses have shown differential sensitivities to amicarbazone applications. For example, amicarbazone may be safely applied at 0.2 kg ha⁻¹ to tall fescue but end-users are limited to sequential applications of 0.05 kg ha⁻¹ on creeping bentgrass because of turfgrass injury (Anonymous 2012). Furthermore, McCullough et al. (2010) noted amicarbazone phytotoxicity was increased with elevated temperatures from 10 to 30 °C on several cool-season grasses, including annual bluegrass, creeping bentgrass, Kentucky bluegrass, and perennial ryegrass. It was suggested that amicarbazone use for annual bluegrass control in cool-season turf may be limited to spring applications, due to increased injury potential from summer applications.

In warm-season turfgrasses, POST application of amicarbazone at 147 to 490 g ha⁻¹ may be used in actively growing bermudagrass and seashore paspalum (Anonymous 2012). Presently, amicarbazone is registered for use in most major turfgrasses (Anonymous 2012) but research is

lacking regarding the use of amicarbazone in seashore paspalum during dormancy or spring green-up.

Although amicarbazone has potential for POST annual bluegrass control, limited research has been conducted on parameters attributed to selectivity of the herbicide for use in turfgrass. Moreover, physiological effects of temperature on amicarbazone behavior in warm- and cool-season grasses have received limited investigation. Amicarbazone also has potential to use in seashore paspalum but application rates, timings, and regimens for turf safety and annual bluegrass control have received limited investigation.

Objective

The objectives of this research were to investigate: (1) absorption, translocation, and metabolism of ^{14}C -amicarbazone in annual bluegrass, creeping bentgrass, and tall fescue, (2) the influence of temperature on injury and growth response of annual bluegrass, bermudagrass, and tall fescue to amicarbazone, (3) physiological effects of temperature on absorption, translocation, and metabolism of ^{14}C -amicarbazone in annual bluegrass, bermudagrass, and tall fescue, (4) bermudagrass and seashore paspalum tolerance to amicarbazone applications for annual bluegrass control during spring green-up, (5) bermudagrass and seashore paspalum growth responses following amicarbazone applications during summer.

Organization

This dissertation consists of introduction and literature review, three research manuscripts, and overall conclusions. Chapter 1 consists introduction and literature review concerning annual bluegrass, chemical control of annual bluegrass in turfgrass, annual bluegrass

control in seashore paspalum, and amicarbazone. Chapter 2 is a manuscript published in the journal of *Weed Science*. It is entitled “Absorption, translocation, and metabolism of amicarbazone in annual bluegrass, creeping bentgrass, and tall fescue”. Chapter 3 is a manuscript accepted by the journal of *Pest Management Science*. It is entitled “Physiological effects of temperature on turfgrass tolerance to amicarbazone.” Chapter 4 is a manuscript submitted to the journal of *Weed Technology*. It is entitled “Seashore paspalum tolerance amicarbazone at various seasonal application timings.” Chapter 5 is a concluding chapter of this dissertation.

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CHAPTER 2

ABSORPTION, TRANSLOCATION, AND METABOLISM OF AMICARBAZONE IN
ANNUAL BLUEGRASS, CREEPING BENTGRASS, AND TALL FESCUE¹

¹J. Yu, P.E. McCullough, and W.K. Vencill. 2013. *Weed Science*. 61:217-221.
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Abstract

Amicarbazone controls annual bluegrass (*Poa annua* L.) in cool-season turfgrasses but physiological effects that influence selectivity have received limited investigation. The objective of this research was to evaluate uptake, translocation, and metabolism of amicarbazone in these species. Annual bluegrass, creeping bentgrass (*Agrostis stolonifera* L.), and tall fescue (*Festuca arundinacea* L.) required <3, 56, and 35 h to reach 50% foliar absorption, respectively. At 72 h after treatment (HAT), annual bluegrass and creeping bentgrass translocated 73 and 70% of root-absorbed ¹⁴C to shoots, respectively, while tall fescue only distributed 55%. Annual bluegrass recovered ≈50% more root-absorbed ¹⁴C in shoots than creeping bentgrass and tall fescue. Creeping bentgrass and tall fescue metabolism of amicarbazone was ≈ 2-fold greater than annual bluegrass from 1 to 7 d after treatment (DAT). Results suggest greater absorption, more distribution, and less metabolism of amicarbazone in annual bluegrass, compared to creeping bentgrass and tall fescue, could be attributed to selectivity of postemergence applications.

Introduction

Annual bluegrass (*Poa annua* L.) is a problematic weed that reduces aesthetics and functionality of cool-season turfgrasses (Beard 1970; Sprague and Burton 1937). Compared to most turf species, annual bluegrass has a lighter green color, coarser leaf texture, and produces unsightly seedheads that reduce turfgrass quality (Lush 1989). Additionally, annual bluegrass has poor disease, heat, and drought tolerances that may increase requirements for water, fungicides, and intensive management in successful turfgrass culture (Beard 1970; Lush 1989).

Preemergence herbicides often provide erratic annual bluegrass control and postemergence (POST) herbicides have limitations on selectivity in cool-season turfgrasses (Callahan and McDonald 1992; Juska and Hanson 1967). Bispyribac-sodium is an acetolactate synthase inhibitor with potential for controlling annual bluegrass in cool-season grasses but applications are limited to spring and early summer to maximize efficacy for turfgrass tolerance and control (Lycan and Hart 2006). Ethofumesate, mesotrione, and sulfosulfuron may control immature annual bluegrass, but inconsistent efficacy and turf injury limit potential for use of these herbicides in cool-season grasses (Jones and Christians 2007; Lycan and Hart 2004; Lycan et al. 2005; Meyer and Branham 2006).

Amicarbazone is a triazolinone herbicide used for weed control in corn (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) (Dayan et al. 2009; Philbrook et al. 1999; Seeruttun et al. 2008). Amicarbazone inhibits photosynthesis and phytotoxic symptoms on susceptible species are similar to triazines including chlorosis, stunted growth, and necrosis of leaves (Senseman 2007). Recently, researchers noted amicarbazone has potential to selectively control annual bluegrass in turfgrasses (Dayan et al. 2009; Perry et al. 2011; McCullough et al. 2010). Injury of cool-season turfgrasses from amicarbazone may increase with elevated temperatures from 10 to

30 °C but spring applications can be effective for selectively controlling annual bluegrass (McCullough et al. 2010). Amicarbazone may be safely applied at 0.2 kg ai ha⁻¹ to tall fescue but end-users are limited to sequential applications of 0.05 kg ha⁻¹ on creeping bentgrass due to turfgrass injury (Anonymous 2012).

Although amicarbazone has potential for POST annual bluegrass control, limited research has been conducted on parameters attributed to selectivity of the herbicide for use in cool-season turfgrass. Thus, comprehensive investigations are required to evaluate amicarbazone physiology in turfgrass to help maximize efficacy for annual bluegrass control. The objective of this research was to evaluate absorption, translocation, and metabolism of amicarbazone in annual bluegrass, creeping bentgrass, and tall fescue.

Materials and Methods

Foliar Absorption and Translocation. Two separate experiments were conducted in Athens, GA from October to December 2011. Single tillers of annual bluegrass, ‘Pennncross’ creeping bentgrass, and ‘Talladega’ tall fescue were transplanted from field samples in Griffin, GA in pots (1.9 cm diam and 20 cm deep) filled with sand:peat moss (80:20 v/v). Annual bluegrass was an indigenous biotype taken from fields without a history of resistance to photosynthesis inhibiting herbicides. After resuming active growth in a greenhouse, grasses were placed in a growth chamber (Environmental Growth Chambers[®], P.O. Box 407, Chagrin Falls, OH 44022) set for 25/20 °C day/night with 470 μmol m⁻² s⁻¹, and photoperiod of 12 h. Plants were watered to prevent wilting and trimmed as needed with shears to maintain 5 cm heights. Grasses were allowed to produce three to five new tillers prior to treatments and plants were selected based on size and population uniformity.

A broadcast application of non-labeled amicarbazone (70WDG, Arysta LifeScience, Suite 150 Cary, NC 27513) was made at $0.2 \text{ kg ai ha}^{-1}$ to grasses in a spray chamber at 374 L ha^{-1} . Immediately after the broadcast application, a $2\text{-}\mu\text{l}$ droplet of spotting solution containing a total of 150 Bq of ^{14}C -amicarbazone (uniformly ring-labeled [specific activity, $39.6 \text{ mCi mmol}^{-1}$, 99.1% radiochemical purity]) was applied to first fully developed leaf. Formulated amicarbazone was added to spotting solution to simulate $0.2 \text{ kg ai ha}^{-1}$ at 374 L ha^{-1} . Nonionic surfactant (NIS) (Activator 90[®], Loveland Industries, Greeley, CO 80631) was added to the spotting solution at 0.125% v/v to facilitate deposition of the droplets on leaves.

Grasses (shoots + roots) were harvested at 3, 6, 12, 24, 48, and 72 h after treatment (HAT). Plants harvested at 24, 48, and 72 HAT were sectioned into treated leaf, non-treated shoots, and roots. Unabsorbed ^{14}C -amicarbazone was removed by swirling the treated leaf in a 20 ml scintillation vial (1654 High Hill Road, Swedesboro, NJ 08085) with 2 ml of 10% methanol solution followed by rinsing with an additional 2 ml of methanol solution. The plant segments were oven dried at $65 \text{ }^{\circ}\text{C}$ for 48 h and combusted in a biological oxidizer (Harvey Biological Oxidizer[®], OX-500, R. J. Harvey Instrument Corp., 11 Jane Stree, Tappan, NY 10983). Radioactivity in the oxidized samples was analyzed by Liquid Scintillation Counter (LSC) (Beckman LS 6500[®], Beckman Coulter Inc., Fall River, MA 02720). Percent foliar absorption was determined by dividing the total ^{14}C recovered in plants by the amount of ^{14}C applied.

Experiment design was completely randomized with four replications and the experiment was repeated. Data were subjected to analysis of variance (ANOVA) with SAS (100 SAS Campus Dr., Cary, NC 27513) and significance of main effects was determined at the 0.05 probability level. Means were separated using Fisher's Protected LSD test at $\alpha = 0.05$.

Absorption data was analyzed with regression analysis, $Y = B(0) + B [1]X + B(2)X^2$. Time required to achieve 50% foliar absorption (t_{50}) was determined for each species. Experiment by treatment interactions were not detected, and thus, results were pooled over experiments.

Root-Applied Absorption and Translocation. Two experiments were conducted in Athens, GA from October to December 2011 with aforementioned plant material. Once grasses had produced three to five new tillers, soil was washed from roots and grasses were placed in a hydroponic tank containing 3 L of half-strength Hoagland solution (Hoagland and Arnon 1950). Roots were submerged in solution by placing grasses through holes in a styrofoam board (Williams Foam, 12961 San Fernando Road, Sylmar, CA 91342) that completely covered the 5-L tank. Sides of the tank were covered with aluminum foil to shield roots from light and an aquarium pump (Shkerry Auqa[®], Shanghai Uni-Aqua Co., Ltd, Chang Shou Road, Shanghai 200042, China) was used to provide plant roots with oxygen. Plants were acclimated in the solution for 7 d.

After acclimation, plants were placed into 50 ml plastic tubes filled with 25 ml half strength Hoagland solution spiked with 26.7 kBq L⁻¹ of ¹⁴C-amicarbazone. Nonlabeled amicarbazone was added to the solution to bring the surface application rate to 0.2 kg ai ha⁻¹. Tubes were covered with aluminum foil to prevent root exposure to light and grasses were suspended in tubes using cotton balls at the base of shoots.

Grasses were harvested at 72 HAT and sectioned to roots and shoots. Roots were rinsed thoroughly with 10% methanol solution and then blotted on a towel. Plants were then oven dried at 60 °C for 48 h, weighed, and oxidized with aforementioned methods. Radioactivity was quantified with LSC and presented as radioactivity per gram of dry weight. Radioactivity per dry weight for total plant was converted with the following equation:

Total ^{14}C recovery = (radioactivity in shoots + radioactivity in roots)/(weight of shoots + weight of roots).

Experimental design was completely randomized with six replications. Significance of main effects was determined using ANOVA in SAS (SAS Institute Inc., Cary NC 27513). Means were separated using Fisher's Protected LSD test at $\alpha = 0.05$. Experiment by treatment interactions were not detected, and thus, experiments were combined.

Metabolism Experiments. Two experiments were conducted in Griffin, GA from July to August 2012. Annual bluegrass, creeping bentgrass, and tall fescue plant material was prepared and grown with aforementioned methods and placed in a growth chamber set for 25/20 °C (day/night) with a 12 hour photoperiod of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After acclimation for one week, plants were treated with a broadcast application of nonlabeled amicarbazone (Amicarbazone, 70WDG herbicide, Arysta LifeSciences, Cary, NC 27513) at 0.2 kg ha^{-1} with a CO_2 -pressured sprayer (Tee Jet[®], Spraying Systems Co., Wheaton, IL 60189-7900). Immediately after broadcast applications, approximately 1.7 kBq of ^{14}C -amicarbazone containing 0.125% v/v NIS was spotted on fully expanded adaxial surface of single leaf of each grass. Treated plants were harvested at 1, 3, and 7 d after treatments (DAT). Unabsorbed ^{14}C was washed off the treated leaf using methods previously described. Treated leaves were stored at -10 °C in a freezer immediately after harvesting.

Individual leaves were placed in 1.5 ml microcentrifuge tubes (Eppendorf, Fisher Scientific, Fair Lawn, NJ 07410) and ground with liquid nitrogen. Tubes with ground leaf tissue were filled with 0.5 ml of hexane: isopropanol: acetic acid (70:29:1). Tubes were placed in water sonication for 45 min. Samples were then centrifuged for 5 min and solution was transferred to

separate tubes. This procedure was conducted three times with fresh solvent added to ground tissue and extraction solutions were combined in a 1.5 ml tube. Residues in tubes were air dried and oxidized to test extraction efficiency.

Extraction solvent was then placed in glass vials and heated to 55 °C under a fume hood to evaporate the solvent. Samples were allowed to cool and then resuspended in 1 ml of dichloromethane. Subsequently, a 500 µl aliquot was spotted on 20 cm length silica gel thin layer chromatography (TLC) plates and developed to 16 cm with ethyl acetate: dichloromethane: acetic acid (20:70:2 v/v/v). TLC plates were then air-dried, and radioactive trace peaks were determined by scanning plates with a radiochromatogram scanner (Bioscan 2000, Bioscan, Inc., 4590 MacArthur Blvd., Washington, DC 20007). Stock radiolabeled herbicide solution was dissolved in 500 µl of dichloromethane, developed on TLC plates, and the parent herbicide was identified at R_f 0.55. Peaks in radioactivity on TLC plates were identified using Laura Chromatography Data Collection and Analysis Software[®] (LabLogic Systems, Inc. 1040 E Brandon Blvd Brandon, FL 33511-5509) connected to the scanner. Metabolites were classified in three groups including parent herbicide, total percentage of metabolites more polar than the parent herbicide, and total percentage of metabolites less polar than the parent herbicide. Data were subjected to analysis of variance (ANOVA) and means were separated using Fisher's Protected LSD test at $\alpha = 0.05$. Experiment by treatment interactions were not detected, and thus experiments were combined.

Results and Discussion

Foliar Absorption and Translocation. Foliar uptake generally increased from 3 to 72 HAT and ranged 56 to 74%, 19 to 59%, and 25 to 59% for annual bluegrass, creeping bentgrass, and tall

fescue, respectively (Figure 2.1). From regression analysis, annual bluegrass, creeping bentgrass, and tall fescue required <3, 56, and 35 h to absorb 50% of the applied herbicide, respectively (Table 2.1). Results suggest annual bluegrass has faster and more foliar absorption of amicarbazone than creeping bentgrass and tall fescue. Foliar uptake of herbicides is related to leaf properties such as cuticle thickness, epicuticular waxes, leaf maturity, and stomata numbers (Chachalis et al. 2001; Hess 1985; Kalnay and Glenn 2000; Sanyal et al. 2006; Wanamarta and Penner 1989). Greater foliar absorption of amicarbazone in annual bluegrass, compared to creeping bentgrass and tall fescue, could be attributed to these properties and may influence differential tolerance levels among species.

At 24 and 48 HAT, annual bluegrass distributed more foliar absorbed ^{14}C to roots (37 and 26%) than creeping bentgrass (12 and 20%) and tall fescue (21 and 15%, respectively) (Table 2.2). Conversely, tall fescue retained more foliar-absorbed ^{14}C in the treated leaf than annual bluegrass and creeping bentgrass at all harvesting timings. From 24 to 72 HAT, the treated leaf of tall fescue retained 62 to 75% of the total ^{14}C recovered, whereas annual bluegrass and creeping bentgrass only had 47 to 55% and 48 to 59% in treated leaves, respectively. By 72 HAT, 30% of foliar-absorbed ^{14}C was found in nontreated shoots of creeping bentgrass, but only 15% was found in nontreated shoots of tall fescue. Generally, ^{14}C concentrations increased in the treated leaf of all grasses at 48 and 72 HAT compared to 24 HAT, which could be from greater foliar penetration at later timings. Delayed herbicide penetration has shown to reduce the rate of herbicide translocation from the treated leaf compared to species with faster foliar uptake (Kalnay and Glenn 2000). Greater herbicide translocation to annual bluegrass roots could increase herbicidal effects in meristematic tissues compared to creeping bentgrass and tall fescue.

Root Absorption and Translocation. At 72 HAT of root applications, annual bluegrass and creeping bentgrass distributed 73 and 70% of the total ^{14}C recovered to shoots, respectively, while tall fescue recovered only 55% in shoots (Table 2.3). Conversely, tall fescue retained 45% of total ^{14}C recovered in roots and was greater than annual bluegrass and creeping bentgrass recoveries of 27 and 30%, respectively. The amount of ^{14}C recovered in roots (Bq g^{-1}) was similar in the three species but annual bluegrass had $\approx 50\%$ more radioactivity recovered in shoots than creeping bentgrass and tall fescue. Similarly, total ^{14}C recovery of annual bluegrass was significantly greater than creeping bentgrass and tall fescue measuring 261, 197, and 192 Bq g^{-1} , respectively.

Results suggest annual bluegrass may distribute more root-absorbed amicarbazone to shoots than creeping bentgrass and tall fescue. Differences in translocation of amicarbazone from roots to shoot meristematic regions may affect the efficacy of root-applied amicarbazone and are consistent with application placement experiments. Perry et al. (2011) noted soil-applied amicarbazone controlled annual bluegrass 57 and 100%, respectively, at 1 and 3 week after treatment (WAT). In contrast, the researchers noted foliar applications had slower activity and controlled annual bluegrass 34 and 93%, respectively, at 1 and 3 WAT. Results suggest annual bluegrass may respond quicker to amicarbazone when absorbed by roots compared to shoots.

Metabolism. Extraction of ^{14}C was efficient as $< 10\%$ of absorbed ^{14}C -amicarbazone was recovered in plant residues (data not shown). The R_f value of amicarbazone was 0.55 and two major metabolites were detected at R_f values of 0.4 and 0.9. Another polar metabolite was detected at 0.15 but was generally $< 10\%$ of total metabolites recovered. Parent herbicide recovery declined from 1 to 7 DAT, suggesting metabolism increased with time in all species

(Table 2.4). From 1 to 7 DAT, metabolism of ^{14}C -amicarbazone differed among species but annual bluegrass recovered approximately twice as much parent herbicide than creeping bentgrass and tall fescue.

At 1 DAT, annual bluegrass, creeping bentgrass, and tall fescue had 49, 17, and 25% parent herbicide recovered, respectively, and levels were comparable at 3 DAT (Table 2.4). By 7 DAT, parent herbicide recovery declined to 27% in annual bluegrass but was greater than creeping bentgrass and tall fescue recoveries of 11 and 14% of total metabolites, respectively.

Polar metabolite recovery was inconsistent across species at 1 and 3 DAT but creeping bentgrass and tall fescue recovery was \approx twofold greater than annual bluegrass at 7 DAT. Nonpolar metabolite recovery was also significantly different in creeping bentgrass and tall fescue compared to annual bluegrass. At 1 DAT, nonpolar metabolite recovery in creeping bentgrass and tall fescue was \approx twofold greater than annual bluegrass (Table 2.4). After 3 and 7 DAT, polar metabolites measured 38 and 51% of the total recovered in creeping bentgrass and tall fescue, respectively, and were significantly greater than 26% recovery of annual bluegrass. After 3 and 7 DAT, nonpolar metabolite recovery in annual bluegrass was generally similar to creeping bentgrass but tall fescue recovered significantly more metabolites than these species.

Differential species metabolism has been reported as the mechanism of selectivity in several herbicides used in grass crops. McCullough et al. (2009) noted differential metabolism levels of bispyribac-sodium could be attributed to selectivity for annual bluegrass control in creeping bentgrass and perennial ryegrass (*Lolium perenne* L.). Olson et al. (2000) found metabolic rate of sulfosulfuron (MON 37500) in wheat (*Triticum aestivum* L.) was greater than downy brome (*Bromus tectorum* L.) and wild oat (*Avena fatua* L.). In other experiments, tolerance of feral rye (*Secale cereale* L.) to imazamox was accredited to greater metabolism than

a sensitive species, jointed goatgrass (*Aegilops cylindrica* Host.) (Pester et al. 2001).

In conclusion, amicarbazone selectivity for annual bluegrass control in cool-season turfgrasses could be attributed to differential levels of absorption, translocation, and metabolism. Annual bluegrass appears to have more absorption and translocation of foliar and root-applied amicarbazone than creeping bentgrass and tall fescue. Amicarbazone appears to be readily taken up by roots and translocated to shoots of annual bluegrass in greater concentrations than creeping bentgrass and tall fescue. Additionally, annual bluegrass appears to have less metabolism of amicarbazone than creeping bentgrass and tall fescue which could influence differential tolerance levels of herbicidal effects on these species.

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Table 2.1. Predicted amount of time required to achieve 50% foliar absorption for three grasses treated with ^{14}C -amicarbazone in two combined growth chamber experiments, 2011, Athens, GA.

Time to Reach 50% Foliar Absorption		
Annual Bluegrass	Creeping Bentgrass	Tall Fescue
----- HAT ^a -----		
<3	56	35
Equations		
$r^2 = 0.21$ $y = 55.86 - 0.35x + 0.009x^2$	$r^2 = 0.49$, $y = 22.9 + 0.26x + 0.004x^2$	$r^2 = 0.38$ $y = 31.2 + 0.68x - 0.004x^2$

^aHAT = hours after treatment

Table 2.2. Translocation of foliar-applied ^{14}C -amicarbazone in annual bluegrass, ‘Penncross’ creeping bentgrass, and ‘Talladega’ tall fescue in two combined growth chamber experiments, 2011, Athens, GA. Different letters indicate significant differences according to Fisher’s Protected LSD test at $\alpha = 0.05$ by column.

Species	Distribution of Recovered ^{14}C in Plant Tissue (HAT) ^a								
	24			48			72		
	Treated Leaf	Nontreated Shoots	Roots	Treated Leaf	Nontreated Shoots	Roots	Treated Leaf	Nontreated Shoots	Roots
	----- % of Absorbed ^{14}C -----								
Annual Bluegrass	47 b	16 b	37 a	51 b	23 a	26 a	55 b	23 b	22 a
Creeping Bentgrass	48 b	40 a	12 c	59 a	21 a	20 b	50 b	30 a	20 a
Tall Fescue	63 a	16 b	21 b	62 a	23 a	15 c	75 a	15 c	10 b

^aHAT = hours after treatment.

Table 2.3. Distribution and specific radioactivity of plant parts at 72 h after root applications of ^{14}C -amicarbazone to three grasses in two combined growth chamber experiments, 2011, Athens, GA. Different letters indicate significant differences according to Fisher's Protected LSD test at $\alpha = 0.05$.

	^{14}C Distribution		Specific Radioactivity		
	Roots	Shoots	Roots	Shoots	Total ^a
	—— % of Absorbed ——		—————Bq/g dry wt—————		
Annual Bluegrass	27 b	73 a	250 a	290 a	261 a
Creeping Bentgrass	30 b	70 a	230 a	173 b	197 b
Tall Fescue	45 a	55 b	201 a	198 b	192 b

^aTotal plant values = (radioactivity in roots + radioactivity in shoots) / (weight of roots + weight of shoots) for each individual plant.

Table 2.4. Metabolism of foliar-applied ^{14}C -amicarbazone in annual bluegrass, creeping bentgrass, and tall fescue at 1, 3, and 7 d after treatment in two combined experiments, 2012, Griffin, GA. Different letters indicate significant differences according to Fisher's Protected LSD test at $\alpha = 0.05$ by column.

Species	Metabolites								
	1 DAT ^a			3 DAT			7 DAT		
	Polar ^b	Parent	Nonpolar	Polar	Parent	Nonpolar	Polar	Parent	Nonpolar
	----- % of total -----								
Annual Bluegrass	26b	49a	25b	35a	44a	21b	31b	27a	42a
Creeping Bentgrass	38a	17b	45a	47a	23b	30ab	51a	11b	38a
Tall Fescue	32ab	25b	43a	41a	24b	35ab	60a	14b	26b

^aDAT = days after treatment

^bMetabolites: Polar, percentage sum of metabolites more polar than amicarbazone; Nonpolar, percentage sum of metabolites less polar than amicarbazone; and Parent, percentage sum of chemicals have similar polarity to amicarbazone.

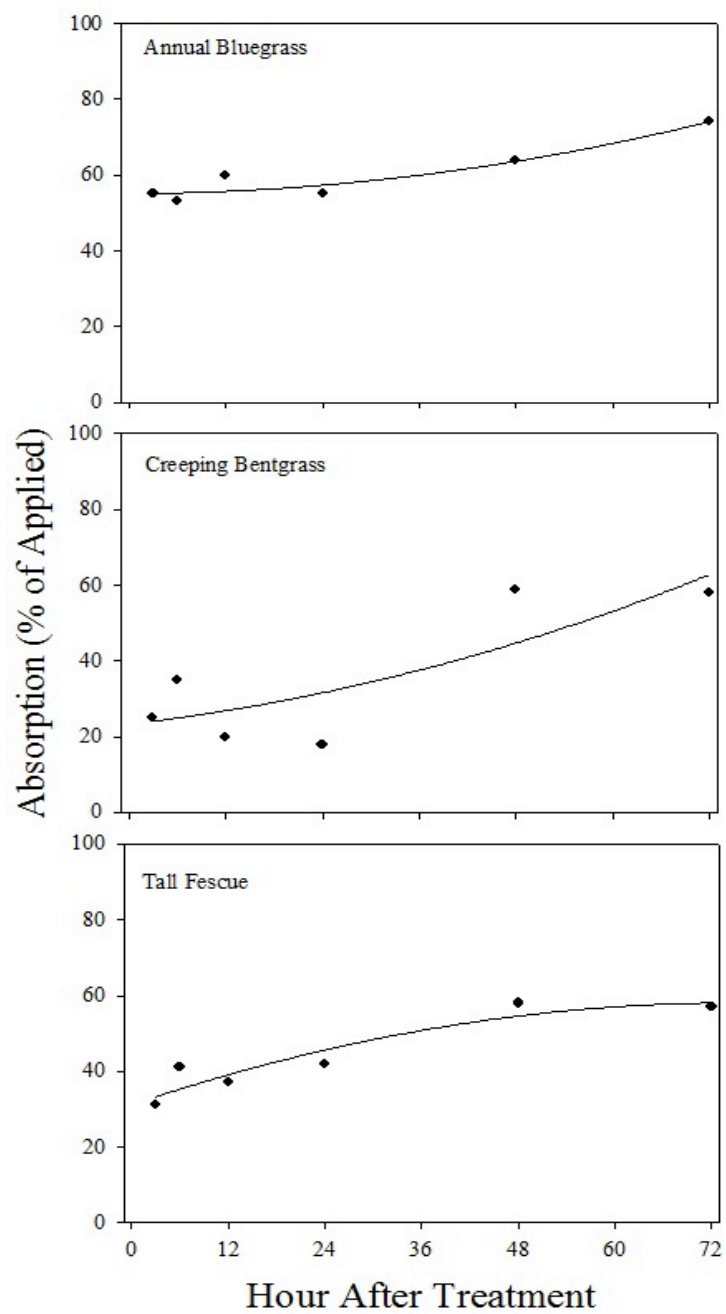


Figure 2.1. Absorption of foliar applied ^{14}C -amicarbazone in two combined growth chamber experiments, 2011, Athens, GA.

CHAPTER 3

PHYSIOLOGICAL EFFECTS OF TEMPERATURE ON TURFGRASS TOLERANCE TO
AMICARBAZONE¹

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Abstract

Amicarbazone effectively controls annual bluegrass (*Poa annua* L.) in bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burt-Davy) and tall fescue (*Festuca arundinacea* Schreb.) with spring applications, but summer applications may excessively injure tall fescue. The objective of this research was to investigate physiological effects of temperature on amicarbazone efficacy, absorption, translocation, and metabolism in annual bluegrass, bermudagrass, and tall fescue. At 25/20 °C (day/night), annual bluegrass absorbed 58 and 40% more foliar applied amicarbazone than bermudagrass and tall fescue, respectively, after 72 h. Foliar absorption increased at 40/35 °C in all species, compared to 25/20 °C, and tall fescue had similar absorption to annual bluegrass at 40/35 °C. At 6 DAT, annual bluegrass metabolized 54% of foliar applied amicarbazone, while bermudagrass and tall fescue metabolized 67 and 64%, respectively. Tall fescue has greater tolerance to amicarbazone than annual bluegrass at moderate temperatures (\approx 25/20 °C) due to less absorption and greater metabolism. However, tall fescue susceptibility to amicarbazone injury at high temperatures (40/35 °C) results from enhanced herbicide absorption compared to lower temperatures (25/20 °C). Bermudagrass is more tolerant to amicarbazone than annual bluegrass and tall fescue due to less herbicide absorption, regardless of temperature.

Introduction

Annual bluegrass (*Poa annua* L.) is a problematic weed that reduces aesthetics and functionality of cool-season turfgrass (Beard 1970). Compared to most turfgrasses, annual bluegrass has a light green color, shallow root system, and coarse leaf texture (Lush 1989; Sprague and Burton 1937). Annual bluegrass often creates unsightly patches in turf stands due to prolific seedhead production and poor tolerances to disease, drought, and traffic (Beard et al. 1978; Lush 1989). As a result, turfgrass infested with annual bluegrass requires more intensive management to maintain acceptable quality (Beard 1970; Lush 1989; Beard 1973).

Preemergence herbicides often provide inconsistent annual bluegrass control, due to poor efficacy or presence of perennial biotypes that are not controlled from applications (Callahan and McDonald 1992). Postemergence herbicides have limitations on selectivity in cool-season turfgrasses, and plant growth regulators only suppress annual bluegrass growth (Callahan and McDonald 1992; McCarty et al. 2005; McCullough et al 2009). Bispyribac-sodium is an acetolactate synthase inhibitor that provides postemergence annual bluegrass control in creeping bentgrass (*Agrostis stolonifera* L.) and perennial ryegrass (*Lolium perenne* L.) (Anonymous 2010; Lycan and Hart 2006; McCullough and Hart 2013). However, application timings of these herbicides are limited to spring or early summer to optimize control and turfgrass tolerance (Lycan and Hart 2006). Ethofumesate and mesotrione may control young annual bluegrass, but erratic control and turf injury often limit potential for successful applications in cool-season turfgrasses (Jones and Christians 2007; Meyer and Branham 2006).

Amicarbazone, 4-amino-*N*-tert-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide, is a triazolinone herbicide that controls weeds by inhibiting photosynthesis, similar to triazines (Dayan et al. 2009; Senseman 2007). Amicarbazone has demonstrated PRE and

POST activity in corn (*Zea mays* L.) and sugar cane (*Saccharum officinarum* L.) fields for controlling a broad spectrum of weeds such as common lambsquarters (*Chenopodium album* L.), common cocklebur (*Xanthium strumarium* L.), velvetleaf (*Abutilon theophrasti* Medik.), and marmaladegrass [*Brachiaria plantaginea* (Link) Hitch.] (Philbrook et al. 1999). Recently, researchers noted amicarbazone has potential for annual bluegrass control in cool-season turfgrass but applications must be made in spring to optimize efficacy (Perry et al. 2011; McCullough et al. 2010). McCullough et al. (2010) noted amicarbazone phytotoxicity was increased with elevated temperatures from 10 to 30 °C on several cool-season grasses, including annual bluegrass, creeping bentgrass, Kentucky bluegrass (*Poa pratensis* L.), and perennial ryegrass. It was suggested that amicarbazone use for annual bluegrass control in cool-season turf may be limited to spring applications, due to increased injury potential from summer applications.

Bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burt-Davy) is a popular warm-season turfgrass species widely used on lawns, roadsides, parks, athletic fields, golf courses, and other areas where close mowing and dense turf are desired (Christians 2004). Bermudagrass is highly tolerant to amicarbazone and may be treated at rates that are double or triple the recommendations for tall fescue (Anonymous 2012). Moreover, bermudagrass may be treated in spring or summer with less potential for injury than tall fescue when temperatures exceed 30 °C (personal observation). Thus, practitioners have greater potential to control annual bluegrass with amicarbazone in bermudagrass due to higher use rates and seasonal application timing flexibility compared with tall fescue.

Selective herbicides for POST annual bluegrass control in tall fescue are limited and amicarbazone represents an important chemistry for end-users. However, a significant limitation

to amicarbazone use in tall fescue is the effects of high temperatures (>30 °C) on turf injury. Physiological effects of temperature on amicarbazone behavior in warm and cool-season grasses have received limited investigation. The objectives of this research were to evaluate (1) the influence of temperature on injury and growth response of annual bluegrass, bermudagrass, and tall fescue to amicarbazone and (2) physiological effects of temperature on absorption, translocation, and metabolism of ¹⁴C-amicarbazone in annual bluegrass, bermudagrass, and tall fescue.

Materials and Methods

Temperature-Dose Response Experiments. Two greenhouse experiments were conducted and repeated over time at the University of Georgia in Griffin, GA from July to September 2013. Annual bluegrass, ‘Titan’ tall fescue, and ‘Princess-77’ bermudagrass were seeded in containers (3.8 cm diam and 20 cm deep) filled with sand:peat moss (80:20 v/v). Annual bluegrass seeds were from indigenous biotype harvested from local fields where no photosystem II inhibitors had been previously applied. Annual bluegrass and tall fescue were grown in a greenhouse set for 23/15 °C (day/night) and bermudagrass was grown in a greenhouse set for 32/25 °C. Plants were watered as needed to prevent moisture stress. Grasses were trimmed weekly to 5 cm in height using shears and pots had about five plants each. Grasses were treated with two applications of a 28-7-14 (N:P₂O₅:K₂O) fertilizer (LescoMarcoN, 15885 Sprague Rd, Strongsville, OH 44136) at 24 kg N ha⁻¹.

Grasses selected for treatments were at a three to five tiller stage of growth, and chosen based on the size and population uniformity. Temperatures of the two greenhouses were set for 32/25 and 23/15 °C day/night, respectively. Grasses were then acclimated at respective

temperatures for 3 d prior to herbicide treatment. A broadcast application of amicarbazone (70WDG, Arysta LifeScience, Cary, NC 27513) was applied at 0, 49, 98, 196, 392, 784, and 1568 g ai ha⁻¹ on grasses with a CO₂-pressured sprayer (Tee Jet[®], Spraying Systems Co., Wheaton, IL 60189) calibrated to deliver 374 L ha⁻¹ of spray volume. Grass shoots were not trimmed during the experiments. Plant injury was visually rated at 2 and 4 weeks after treatment (WAT) on a percent scale, where 0 equaled no injury and 100 equaled complete desiccation. Shoots were harvested with shears at 4 WAT to the soil surface, oven-dried at 65 °C for 48 h, and weighed.

Experimental design was a completely randomized split plot with four replications. Main plot factors were two temperature regimens and sub-plot factors were grass species. Data were subjected to analysis of variance (ANOVA) using SAS (100 SAS Campus Dr., Cary, NC 27513). Experiment by treatment interaction was not detected and thus, data were combined for analysis. Dose response data were subjected to a regression analysis, $y = a*[1-\exp(-b*x)]$, and coefficients of correlation as well as standard error values were determined in SigmaPlot (Systat Software, Inc. 1735 Technology Drive, Suite 430 San Jose, CA 95110). From regression analysis, herbicide dose causing 50% injury (I₅₀) and 50% dry shoot weight reduction (GR₅₀) were determined.

Foliar Absorption Experiments. Two experiments were conducted in Griffin, GA in fall 2012. Experiments were repeated over time. Annual bluegrass, bermudagrass, and tall fescue were seeded and grown in the greenhouse as described in previous experiments. Grasses were thinned to one plant per container and placed in growth chambers (Percival Scientific, 505 Research Drive, Perry, IA 50220) set for 25/20 °C (day/night) or 40/35 °C temperatures with

approximately 50% relative humidity and 12 h photoperiod. Irradiance intensity of these lights was $350 \mu\text{mol m}^{-2} \text{s}^{-1}$. Grasses were watered as needed and acclimated in growth chambers for 3 d prior to treatment. A broadcast application of amicarbazone was applied at 196 g ha^{-1} at 374 L ha^{-1} with aforementioned CO_2 pressured sprayers. Immediately after the broadcast application, two 1-mL droplets of ^{14}C -amicarbazone (uniformly ring-labeled [specific activity, $39.6 \text{ mCi mmol}^{-1}$, 99.1% radiochemical purity]) measuring a total of 200 Bq radioactivity was applied to adaxial surface of the fully developed leaf. Formulated amicarbazone was added to the spotting solution to simulate 196 g ha^{-1} at 374 L ha^{-1} or 0.52 mg mL^{-1} . The spotting solution had a nonionic surfactant (Activator 90[®], Loveland Industries, Greeley, CO 80631) included at 0.125% v/v to aid deposition of droplets on leaves.

Plants (root plus shoots) were harvested at 72 h after treatment (HAT). Unabsorbed ^{14}C -amicarbazone was removed by swirling the treated leaf in a 20 ml glass scintillation vial (1654 High Hill Road, Swedesboro, NJ 08085) with 2 ml of 20% methanol solution for 45 s followed by rinsing with additional 2 ml. Plant samples were oven dried at $65 \text{ }^\circ\text{C}$ for 48 h and combusted in a biological oxidizer (Harvey Biological Oxidizer[®], OX-500, R. J. Harvey Instrument Corp., 11 Jane Stree, Tappan, NY 10983). Radioactivity in oxidized samples was quantified using liquid scintillation spectroscopy (Beckman LS 6500[®], Beckman Coulter Inc., Fall River, MA 02720). Percent foliar absorption was calculated by dividing the total ^{14}C recovered in plants by the total amount of ^{14}C applied.

Experimental design was a completely randomized split plot with temperature regimens as main plot effect and grass species as sub-plot effect. Four pots were placed in each temperature regimens as subsamples and regarded as replicates in the analysis. Due to limited chambers available, only two growth chambers were used. Data were subjected to ANOVA with

SAS at the 0.05 probability level. Interactions of foliar absorption by experiment were not detected, and thus data were pooled over experiments for analysis. Interaction of temperature and grass species was detected, and thus all treatments were presented. Treatment means were separated with Fisher's Protected LSD test at $\alpha = 0.05$.

Root Absorption and Distribution Experiments. Two experiments were conducted in Griffin, GA from September to October 2012 using the plant material described previously. Grasses selected for treatments were at a three to five tiller stage of growth and chosen based on size uniformity. Grasses were removed from pots and soil was washed from roots. Plants were acclimated hydroponically in a 4 L plastic tank that was filled with 3 L of half-strength Hoagland solution (Hoagland and Arnon 1950). Plants were placed through 1-cm-diam holes in a 1.2-cm thickness of styrofoam board (12961 San Fernando Road, Sylmar, CA 91342), and roots were suspended in the nutritive solution. Sides of tank were covered with aluminum foil to shield roots from light. An aquarium pump (ShkerryAqua[®], Shanghai Uni-Aqua Co., Ltd, Chang Shou Road, Shanghai 200042, China) was used to constantly provide oxygen to the nutrient solution. Plants were acclimated in the solution for 7 d prior to treatment in a greenhouse set at 23/15 °C.

After acclimation, individual plants were set through cotton balls and placed into 50 ml plastic tubes (5.1 cm² surface area by 15 cm deep) filled with 25 ml half-strength Hoagland solution spiked with 26 kBq L⁻¹ of ¹⁴C-amicarbazone. Grass roots were suspended in the herbicide solution and tubes were covered with aluminum foil. Formulated amicarbazone was added at 0.4 mg mL⁻¹ to simulate a 196 g ha⁻¹ surface application rate. Grasses were placed in growth chambers set for 25/20 °C or 40/35 °C with approximately 50% relative humidity and 12 h photoperiods of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Plants were harvested at 72 HAT and sectioned to roots and shoots. Roots were rinsed thoroughly using 20% methanol solution. Plants were oven dried at 65 °C for 48 h. Plant samples were then oxidized and radioactivity was quantified using the procedure described in foliar-applied absorption experiments.

Absorption data was determined by dividing ^{14}C in plants by the amount ^{14}C -amicarbazone applied in each tube. ^{14}C distribution data were calculated by dividing the radioactivity detected in shoots or roots to the total radioactivity in the entire plant.

Experimental design was completely randomized split-plot with four replications. Main plot factors were two temperature regimens and sub-plot factors were grass species. Significance of main effects was determined using ANOVA in SAS and means were separated with Fisher's Protected LSD test at $\alpha = 0.05$. Main effects by experiment interactions were not detected and thus, data for the experiments were combined for analysis.

Metabolism Experiments. Two experiments were conducted in Griffin, GA in fall of 2012. Annual bluegrass, bermudagrass, and tall fescue were grown in the greenhouse using the methods described previously. Grasses were acclimated in growth chamber for 3 d prior to treatment. After acclimation, plants were sprayed 196 g ha⁻¹ at 374 L ha⁻¹ nonlabeled amicarbazone with a CO₂-pressured sprayer. Immediately after the broadcast applications, two 1-mL droplets of ^{14}C -amicarbazone containing 1.7 kBq of ^{14}C -amicarbazone with 0.125% v/v nonionic surfactant were spotted on first expanded adaxial surface of each grass. Treated leaves were harvested at 6 d after treatment (DAT). This harvest timing and methodology was chosen from previous research on amicarbazone metabolism (Yu et al. 2013). Unabsorbed herbicide was removed using the procedure described previously. Immediately after harvesting, treated leaves

were stored at -20 °C.

Individual leaves were placed in 1.5 ml microcentrifuge tubes (Eppendorf, Fisher Scientific, Fair Lawn, NJ 07410) filled with liquid nitrogen. Leaf tissue was ground with liquid nitrogen in tubes using a pestle. The tubes containing the ground leaf tissue was then filled with 0.5 ml of hexane: isopropanol: acetic acid (70:29:1 v/v/v) and placed in a water sonication (Fisher Scientific 300, Industry Drive, Pittsburgh, PA 15275) for 1 h. Tubes were centrifuged for 5 min and extracted solution was transferred to separated tubes. This extraction procedure was repeated three times with fresh solvent used each time. Extracted solution was combined into a 1.5 ml microcentrifuge tube. Plant residue left in tubes was air-dried, oxidized, and quantified in liquid scintillation spectroscopy to examine the extraction efficacy.

Extracted solution was then transferred into 5-ml reactive vials (Thermo Scientific, 320 Rolling Ridge Drive, Bellefonte PA 16823) and evaporated in a heating block set for 60 °C temperature under a fume hood. After evaporation, samples were resuspended in 1 ml of dichloromethane and spotted on 20 cm length silica gel thin layer chromatography (TLC) plates. The spotted TLC plates were developed using a solvent that is a mixture of ethyl acetate: dichloromethane: acetic acid (10:35:1 v/v/v). TLC plates were then air-dried and scanned using a radiochromatogram scanner (Bioscan 2000, Bioscan, Inc., 4590 MacArthur Blvd., Washington, DC 20007). Radioactive trace peaks were determined using a computer installed Laura Chromatography Data Collection and Analysis Software[®] (LabLogic Systems, Inc. 1040 E Brandon Blvd Brandon, FL 33511-5509).

Parent herbicide was determined by comparing retention factor (R_f) to ¹⁴C-amicarbazone stock solution. Metabolism was quantified by dividing the sum percentage of metabolite radioactivity from the total radioactive peaks (metabolites plus parent). Experimental design was

a completely randomized split-plot with four replications. Main plot effect was temperature, and sub-plot effect was grass species. Main effects were separated using Fisher's Protected LSD test at $\alpha = 0.05$. Main effects of treatment by experiment interactions were not detected, and thus, data from both experiments were combined for analysis.

Results and Discussion

Temperature-Dose Response Experiments. Species by temperature interaction was detected for injury and shoot biomass reduction, and thus, results are presented across temperatures by species (Table 3.1, Fig. 3.1, 3.2). From regression analysis, I_{50} values of annual bluegrass, bermudagrass, and tall fescue measured $>1568 \text{ g ha}^{-1}$ at low temperatures by 2 WAT. However, annual bluegrass and tall fescue I_{50} values at high temperatures measured 146 and 513 g ha^{-1} , respectively, while bermudagrass I_{50} measured $>1568 \text{ g ha}^{-1}$. Similarly, annual bluegrass, bermudagrass, and tall fescue I_{50} values measured $>1568 \text{ g ha}^{-1}$ at low temperatures by 4 WAT. In contrast, annual bluegrass and tall fescue I_{50} values measured 161 and 331 g ha^{-1} , respectively, while bermudagrass I_{50} values measured 1288 g ha^{-1} at high temperatures by 4 WAT.

Temperature had a greater effect on shoot biomass reductions of annual bluegrass and tall fescue than bermudagrass following amicarbazone treatments (Table 3.1, Fig. 3.2). Annual bluegrass and tall fescue GR_{50} values at low temperatures measured 866 and $>1568 \text{ g ha}^{-1}$, respectively, but GR_{50} values were reduced to 147 and 117 g ha^{-1} at high temperatures, respectively. Bermudagrass shoot growth was less affected by amicarbazone than annual bluegrass and tall fescue, and GR_{50} values measured $>1568 \text{ g ha}^{-1}$ at both temperature regimens.

Temperature significantly affects the tolerance of cool-season grasses to amicarbazone. McCullough et al. (2010) reported annual bluegrass, creeping bentgrass, Kentucky bluegrass,

and perennial ryegrass injury was exacerbated by amicarbazone when temperature increased from 10 to 30 °C. It was also noted that cool-season grasses exhibited differential tolerances to amicarbazone, as Kentucky bluegrass and perennial ryegrass were more tolerant than annual bluegrass and creeping bentgrass at 20 °C. Tall fescue is tolerant to amicarbazone labeled use rates when temperatures are <30 °C, and treatments may effectively control annual bluegrass in spring. However, tall fescue sensitivity increases substantially as temperatures increased, and selectivity for annual bluegrass control is diminished under high temperatures.

Bermudagrass exhibited considerably greater tolerance to amicarbazone than tall fescue, despite exacerbated injury and shoot biomass reduction with increased temperatures. Selectivity of amicarbazone for controlling annual bluegrass in bermudagrass could also be diminished under temperatures greater than those evaluated, but effects of the high temperature were more substantial on tall fescue. Differential tolerance levels to amicarbazone under warm temperatures could allow turfgrass managers to suppress tall fescue in polyculture with bermudagrass in summer. Further research is needed to evaluate potential cool-season grass suppression in bermudagrass from amicarbazone rates during summer.

¹⁴C-amicarbazone Foliar Absorption. Temperature by species interaction was detected for foliar absorption, and thus, results are presented across all combinations (Table 3.2). At 72 HAT, bermudagrass and tall fescue had similar foliar absorption of ¹⁴C-amicarbazone, measured 31 and 35% of ¹⁴C applied, respectively. Annual bluegrass had more foliar absorption of ¹⁴C-amicarbazone than bermudagrass and tall fescue at 25/20 °C, and measured 49% of the applied. The increase in temperature from 25/20 to 40/35 °C enhanced foliar absorption of ¹⁴C-

amicarbazone in all grasses. At 40/35 °C, tall fescue had similar foliar absorption to annual bluegrass, averaging 93% of ^{14}C applied, but bermudagrass had only 56% absorption.

Researchers have previously reported greater herbicide phytotoxicity at high temperatures was attributed to increased absorption compared to lower temperature. For example, Masiunas and Weller (1988) noted that potato (*Solanum tuberosum* L.) grown at 24/13 °C exhibited greater phytotoxicity from glyphosate than plants grown at 13/4 °C. It was noted that ^{14}C -glyphosate uptake by potato at 13/4 °C was only 1/3 of the total ^{14}C -glyphosate found in potato grown at 24/13 °C. Similarly, increased absorption of ^{14}C -picloram and ^{14}C -mefluidide at higher temperature, compared to lower temperature regimens, was noted in common cocklebur (*Xanthium pensylvanicum* Wallr.), leafy spurge (*Euphorbia esula* L.), and soybean (*Glycine max* L. Merr.) (Lym and Moxness 1989; McWhorter and Wills 1978).

Foliar uptake and cuticular penetration of herbicides has been reported to vary among species and environmental conditions (Black et al. 1995; Greene and Bukovac 1971; Orbović and Achor 2001). Plant leaf properties such as cuticle (cutin, epicuticular waxes, and pectin), and stomatal and trichome density have a role in influencing foliar absorption of herbicides (Chachalis et al. 2001; Cobb and Reade 2010; Hess 1984; Hess et al. 1974; Sanyal et al. 2006; Wanamarta and Penner 1989). For example, the production of leaf waxes in cauliflower (*Brassica napus* L.) and manna gum (*Eucalyptus viminalis* Labill.) was greater at lower temperatures compared to higher temperatures (Baker 1974; Banks and Whitecross 1971). Temperature effects on stomatal conductance, leaf growth, and the use of adjuvants may also affect foliar herbicide penetration (Ditomaso 1999; Stevens 1993). Differential absorption at various temperature regimens was probably attributed to differences in leaf properties among species and further investigation is warranted to evaluate these morphological characteristics in

turfgrasses.

Root Absorption and Distribution. Species by temperature interactions were not detected for root absorption or distribution, and thus, main effects are presented (Table 3.3). After 72 HAT, root absorption of ^{14}C -amicarbazone was similar for annual bluegrass and tall fescue, averaging 53% of ^{14}C applied. However, bermudagrass had significantly less root uptake, and measured 29% of ^{14}C applied. Physiologically, cool-season grasses have greater water requirements than warm-season grasses (Feldhake et al. 1983; Fu et al. 2004). These differences are likely associated with more root absorption of soil-applied herbicides for cool-season grasses, compared to bermudagrass. Grasses averaged twice as much root absorbed ^{14}C -amicarbazone at 40/35 °C than at 25/20 °C.

All grasses had similar distribution of radioactivity from root absorption, and averaged 84% translocation to shoots. Grasses grown at 40/35 °C averaged 10% more ^{14}C distributed to shoots than at 25/20 °C. Similar increases in acropetal movement were noted following root-absorption of ^{14}C -methiozolin in annual bluegrass and creeping bentgrass grown at 15/10 and 30/25 °C (McCullough et al. 2013). Greater herbicide absorption and translocation at higher temperature may be related to the altered stomatal conductance and increased transpiration rate (Fletcher et al. 2007).

Greater herbicide absorption and translocation detected at high temperature could strengthen herbicidal effects in meristematic regions (Koren and Ashton 1973; Kozlowski et al. 1967; Penner 1971; Sheets 1961), resulting in greater phytotoxicity on grasses. Greater phytotoxicity of atrazine and simazine at higher temperatures was reported due to increased herbicide uptake (Kozlowski et al. 1967; Penner 1971; Vostral et al. 1970; Wax and Behrens

1965). ^{14}C -atrazine uptake by soybean (*Glycine max* L. Merr.) roots increased as temperature increased from 20 to 25 °C (Penner 1971). Similarly, oat (*Avena sativa* L.) and cotton (*Gossypium hirsutum* L.) leaf tissues accumulated more than twice concentration of simazine when plants grown in 37 °C than in 26 °C (Sheets 1961). In other herbicides, Koren and Ashton (1973) noted root absorption of ^{14}C -pyrazone in sugar beet (*Beta vulgaris* L.) at 35 °C was twofold higher than at 18.3 °C after 360 min of herbicide treatment. It was also noted that translocation of ^{14}C -pyrazone into sugar beet shoots was faster at 35 °C than 18.3 °C.

Metabolism. Extraction efficiency of the method used in the study was 90%. The R_f value of amicarbazone was 0.55 and two major metabolites were found at R_f values of 0.4 and 0.9. An insignificant polar metabolite was detected at R_f values of 0.15 but was generally <10% of total metabolites recovered.

At 6 DAT, species by temperature interactions were not detected for total metabolites (Table 3.4). Annual bluegrass metabolized 54% of foliar absorbed ^{14}C -amicarbazone, while bermudagrass and tall fescue metabolized 67 and 64%, respectively. Temperature had a significant effect on amicarbazone metabolism. After 6 DAT, averaged across species, grasses grown at 25/20 °C metabolized 56% foliar applied ^{14}C -amicarbazone, while grasses grown at 40/35 °C metabolized 68%.

Differential herbicide metabolism in grasses has been associated with amicarbazone selectivity in turfgrasses. Yu et al. (2013) reported annual bluegrass had less amicarbazone metabolism than creeping bentgrass and tall fescue from 1 to 7 d after treatment. Selectivity of other chemistries for annual bluegrass control has been attributed to differential metabolism from tolerant grasses. McCullough et al. (2009) noted selectivity of bispyribac-sodium for annual

bluegrass control in creeping bentgrass and perennial ryegrass is a result of less metabolism. Researchers have also noted herbicide metabolism was enhanced with increased temperatures in grasses. Koeppe et al. (2000) reported rimsulfuron selectivity for weed control in maize (*Zea mays* L.) was attributed to metabolism. Maize also had increased metabolism of rimsulfuron at 25 to 30 °C, compared to lower temperatures (Koeppe et al. 2000).

Turfgrass managers may effectively use amicarbazone for annual bluegrass control in bermudagrass and tall fescue in spring. Increased tall fescue injury from summer applications of amicarbazone are likely attributed to effects of temperature on herbicide phytotoxicity. Bermudagrass is more tolerant to amicarbazone than tall fescue, regardless of temperatures, and differential response of amicarbazone in these turfgrasses is influenced by temperature. Bermudagrass foliar uptake was comparable to tall fescue but less than annual bluegrass at low temperatures. Foliar and root uptake increased as temperature increased in all grasses but uptake of amicarbazone in bermudagrass was less than annual bluegrass and tall fescue at high temperatures. Metabolism of amicarbazone increased in all grasses at high temperatures, compared to low temperatures, but annual bluegrass averaged less metabolism than bermudagrass and tall fescue. Overall, physiological effects of temperature on amicarbazone selectivity in turfgrasses are attributed to differential levels of herbicide absorption by roots and shoots.

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Table 3.1. Predicted rates from regression equations to injure annual bluegrass, ‘Princess-77’ bermudagrass, and ‘Titan’ tall fescue 50% (I_{50}) and reduce dry shoot weight 50% from the untreated control (GR_{50}) at 4 week after amicarbazone application, Griffin, GA, 2013. Values represent mean of two greenhouse experiments.^a

Species	Temperature ^b	I_{50}		GR_{50}
		2 WAT	4 WAT	4 WAT
-----g ai ha ⁻¹ -----				
Annual bluegrass	Low	>1568	>1568	866
	High	146	161	147
Bermudagrass	Low	>1568	>1568	>1568
	High	>1568	1288	>1568
Tall fescue	Low	>1568	>1568	>1568
	High	513	331	117
LSD0.05		38	153	343
Equations				
Annual bluegrass	Low	$r^2 = 0.60, Y = 32.64*(1 - \exp(-0.0028*x)), SE = 6.47.$	$r^2 = 0.36, Y = 38.31*(1 - \exp(-0.0028*x)), SE = 10.01.$	$r^2 = 0.36, Y = 50.80*(1 - \exp(-0.0048*x)), SE = 10.01.$
	High	$r^2 = 0.66, Y = 79.58*(1 - \exp(-0.0070*x)), SE = 14.36.$	$r^2 = 0.82, Y = 89.98*(1 - \exp(-0.0051*x)), SE = 11.36.$	$r^2 = 0.43, Y = 75.17*(1 - \exp(-0.0070*x)), SE = 18.75.$
Bermudagrass	Low	$r^2 = 0.89, Y = 65.51*(1 - \exp(-0.0003*x)), SE = 2.88.$	$r^2 = 0.39, Y = 21.19*(1 - \exp(-0.0032*x)), SE = 6.93.$	$r^2 = 0.39, Y = 48953.00*(1 - \exp(-1.68E-7*x)), SE = 6.93.$
	High	$r^2 = 0.73, Y = 63.89*(1 - \exp(-0.0006*x)), SE = 8.67.$	$r^2 = 0.89, Y = 136.64*(1 - \exp(-0.0004*x)), SE = 5.95.$	$r^2 = 0.11, Y = 34.39*(1 - \exp(-0.0067*x)), SE = 23.58.$
Tall fescue	Low	$r^2 = 0.63, Y = 15.99*(1 - \exp(-0.0020*x)), SE = 4.39.$	$r^2 = 0.63, Y = 17.49*(1 - \exp(-0.0026*x)), SE = 4.39.$	$r^2 = 0.63, Y = 48.37*(1 - \exp(-0.0008*x)), SE = 4.39.$
	High	$r^2 = 0.92, Y = 116.83*(1 - \exp(-0.0011*x)), SE = 9.38.$	$r^2 = 0.88, Y = 104.72*(1 - \exp(-0.0020*x)), SE = 11.87.$	$r^2 = 0.38, Y = 76.14*(1 - \exp(-0.0104*x)), SE = 20.38.$

^aAbbreviation: SE = standard error of estimates; WAT = week after treatment.

^bHigh temperature set for 32/25 °C day/night; low temperature set for 23/15 °C day/night.

Table 3.2. Foliar absorption at 72 h after ^{14}C -amicarbazone treatment in three grasses at two temperature regimens, Griffin, GA, 2012. Values represent mean of two growth chamber experiments.

Temperature ^a (day/night)	Species ^b	Absorption % of applied
25/20 °C	Annual bluegrass	49
	Bermudagrass	31
	Tall fescue	35
	LSD _{0.05}	8
40/35 °C	Annual bluegrass	94
	Bermudagrass	56
	Tall fescue	92
	LSD _{0.05}	12
	Species	*
	Temperature (Temp)	*
	Species x Temp	*

^aTemperature regimens and photoperiods lasted 12 h in growth chambers.

^b‘Princess-77’ bermudagrass and ‘Titan’ tall fescue were seeded in pots while annual bluegrass was established from seed of an indigenous biotype to Griffin, GA.

Table 3.3. Absorption and radioactivity distribution at 72 h after root-applied ^{14}C -amicarbazone in three grasses at two temperature regimens, Griffin, GA, 2012. Values represent mean of two growth chamber experiments.

Species ^a	Absorption % of applied	^{14}C Distribution	
		Roots %	Shoots %
Annual bluegrass	56	15	85
Bermudagrass	29	17	83
Tall fescue	50	16	84
LSD _{0.05}	10	NS	NS
Temperature ^b			
25/20 °C	30	21	79
40/35 °C	59	11	89
LSD _{0.05}	9	3	3
Species	*	NS	NS
Temperature (Temp)	*	*	*
Species x Temp	NS	NS	NS

^a‘Princess-77’ bermudagrass and ‘Titan’ tall fescue were seeded in pots while annual bluegrass was established from seed of an indigenous biotype to Griffin, GA.

^bTemperature regimens and photoperiods lasted 12 h in growth chambers.

Table 3.4. Total metabolite recovery in treated leaves of three grasses at 6 d after ^{14}C -amicarbazone treatment, Griffin, GA, 2012. Values represent mean of two growth chamber experiments.

Species ^a	Total Metabolites
	% of ^{14}C recovered
Annual bluegrass	54
Bermudagrass	67
Tall fescue	64
	LSD _{0.05}
	6
Temperature ^b	
25/20 °C	56
40/35 °C	68
	LSD _{0.05}
	5
Species	*
Temperature (Temp)	*
Species x Temp	NS

^aTemperature regimens and photoperiods lasted 12 h in growth chambers.

^b‘Princess-77’ bermudagrass and ‘Titan’ tall fescue were seeded in pots while annual bluegrass was established from seed of an indigenous biotype to Griffin, GA. Plants were multi-tiller and acclimated in growth chambers for 24 h before treatments.

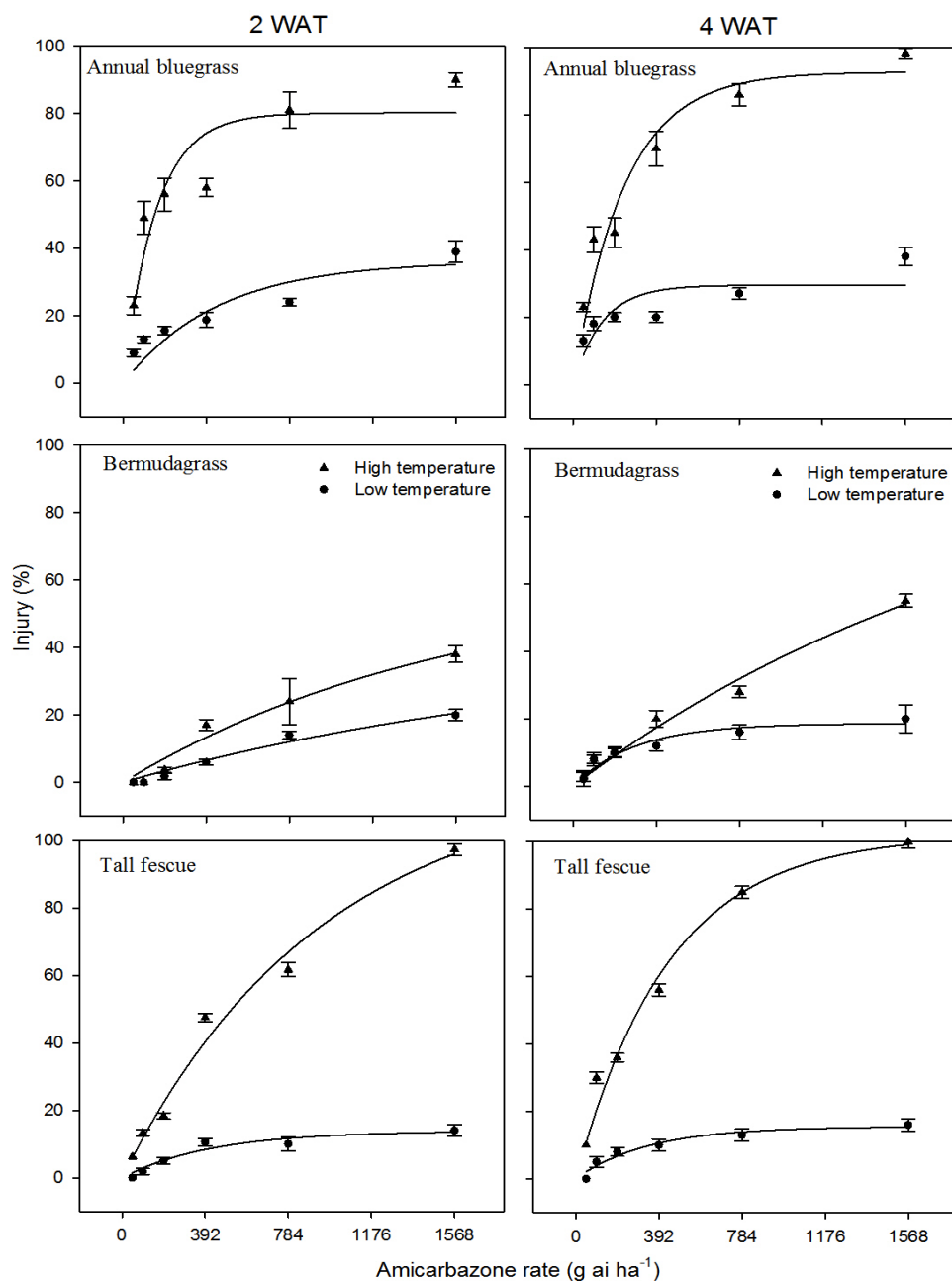


Figure 3.1. Amicarbazone dose response on percent injury in annual bluegrass, bermudagrass, and tall fescue at 2 and 4 wk after treatment. Values represent mean of two greenhouse experiments. Abbreviation: WAT: week after treatment. High temperature set for 32/25 °C day/night; low temperature set for 23/15 °C day/night.

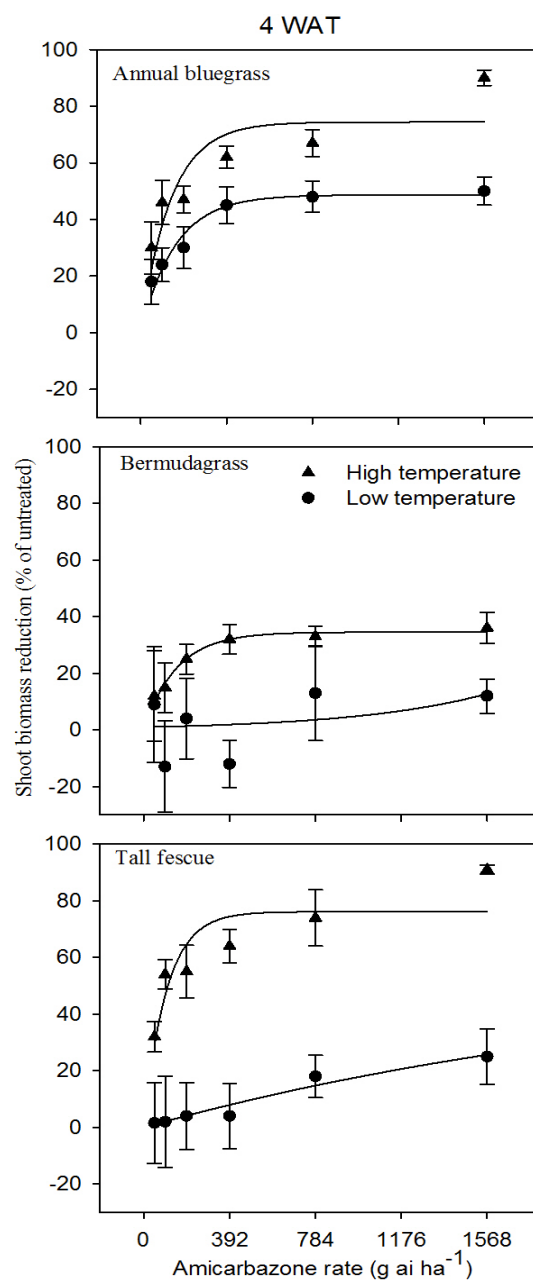


Figure 3.2. Amicarbazone dose response on shoot biomass reduction in annual bluegrass, bermudagrass, and tall fescue at 4 wk after treatment. Values represent mean of two greenhouse experiments. Abbreviation: WAT: week after treatment. High temperature set for 32/25 °C day/night; low temperature set for 23/15 °C day/nigh.

CHAPTER 4
SEASHORE PASPALUM TOLERANCE TO AMICARBAZONE AT VARIOUS SEASONAL
APPLICATION TIMINGS¹

¹Jialin Yu, Patrick E. McCullough, and Mark A. Czarnota. Accepted by *Weed Technology*.
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Abstract

Turfgrass injury from triazines has limited the use of Photosystem II (PS II) inhibitors for weed control in seashore paspalum. Amicarbazone is a new PS II inhibitor with potential safety in seashore paspalum, but the effects of application timing on turf tolerance has received limited investigation. Field experiments were conducted in Griffin, GA to evaluate the tolerance of 'Sea Isle 1' seashore paspalum to amicarbazone applications in winter, spring, and summer. Seashore paspalum had minimal injury (<5%) from amicarbazone treatments (98, 196, and 392 g ai ha⁻¹) applied for annual bluegrass control in winter and spring. By 6 wk after treatment (WAT), amicarbazone at 392 g ha⁻¹ provided 78 and 90% annual bluegrass control in 2013 and 2014, respectively, and was similar to pronamide at 1680 g ai ha⁻¹. Amicarbazone at 196 g ha⁻¹ provided 71% control of annual bluegrass in 2014, but control was poor (<70%) in 2013. Sequential amicarbazone applications at 98 g ha⁻¹ provided poor control in both years by 6 WAT. From six amicarbazone rates (up to 984 g ha⁻¹) applied in summer, seashore paspalum required 510 and <123 g ha⁻¹ for 20% turfgrass injury (I₂₀) and 20% clipping reduction (CR₂₀), respectively, while I₂₀ and CR₂₀ measured >984 g ha⁻¹ for 'Tifway' bermudagrass. Overall, amicarbazone may be safely applied to seashore paspalum in winter, spring, and summer at rates and regimens evaluated. However, seashore paspalum may exhibit shoot growth inhibition up to 4 WAT suggesting end-users should be cautious when using amicarbazone during active growth in summer.

Introduction

Seashore paspalum (*Paspalum vaginatum* Swartz) is a warm-season turfgrass planted in tropical and warm temperate regions for golf courses, sports fields, and lawns (Duncan and Carrow 2000). Seashore paspalum tolerates various abiotic stresses such as drought, traffic, water logging, and low soil pH (Dudeck and Peacock 1985; Trenholm et al. 2000). It is also tolerant to irrigation with salt-laden water and may be grown in coastal areas in place of other species, such as bermudagrass (*Cynodon dactylon* L.) (Dudeck and Peacock 1985; Duncan and Carrow 2000).

A major limitation to seashore paspalum management is susceptibility to herbicide injury. Controlling weeds such as annual bluegrass (*Poa annua* L.) may be difficult in seashore paspalum due to a lack of selective herbicide chemistries. For example, ethofumesate labeled for use in seashore paspalum at low rates (560 g ha⁻¹) but efficacy is often reduced on mature annual bluegrass (Johnson 1983). Moreover, ethofumesate rates required for effective annual bluegrass control may cause excessive (>20%) injury to seashore paspalum (Unruh et al. 2006). Sulfonylureas, such as foramsulfuron, rimsulfuron, and trifloxysulfuron, have potential to effectively control annual bluegrass but often cause unacceptable injury (>20%) to seashore paspalum at labeled use rates (Toler et al. 2007; McCullough et al. 2012). Pronamide is a cell division inhibitor that selectively controls annual bluegrass in seashore paspalum (McCullough et al. 2012). However, annual bluegrass control with pronamide is slow and treatments have potential for lateral movement to susceptible species (Askew 2011; Senseman 2007). Thus, new chemistries are needed for use in seashore paspalum for POST annual bluegrass control that are safe and effective at various application timings.

Amicarbazone is a PS II inhibitor that effectively controls common cocklebur (*Xanthium strumarium* L.), common lambsquarters (*Chenopodium album* L.), and pigweed species (*Amaranthus* spp.) in corn (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) (Philbrook et al. 1999). Phytotoxic symptoms of susceptible plants treated with amicarbazone include stunted growth, tissue chlorosis and necrosis, and eventual plant death (Senseman 2007). Amicarbazone also controls annual bluegrass in turfgrasses and may offer an alternative chemistry for use in species with potential sensitivity to triazines or sulfonyleureas, such as seashore paspalum (Johnston and McCullough 2014).

The limitations on selective POST herbicides available in seashore paspalum also have important implications for resistance management. The introduction of amicarbazone may provide seashore paspalum managers an opportunity to incorporate a PS II inhibitor in weed control programs. Seashore paspalum has shown potential tolerance to amicarbazone applied in spring (Johnston and McCullough 2014), but the effects of application rates, timings, and regimens on turf safety and annual bluegrass control have received limited investigation. The objectives of this research were to evaluate seashore paspalum tolerance to (1) amicarbazone rate and timing for annual bluegrass control in winter and spring and (2) six rates of amicarbazone applied in summer.

Materials and Methods

Winter and Spring Experiments. Research was conducted in Griffin, GA from February to May in 2013 and 2014 on mature stands of ‘TifSport’ bermudagrass and ‘Sea Isle 1’ seashore paspalum. Plots in 2014 were adjacent to plots used in 2013 on both fields. Bermudagrass was grown on a Cecil sandy clay loam (Fine, kaolinitic, thermic Typic Kanhapluduts) with

approximately 2% organic matter. Seashore paspalum was grown on an Appling sandy clay loam with 4.4% organic matter. The pH of both fields was approximately 6.0. Annual bluegrass control was rated in bermudagrass due to minimal populations present in seashore paspalum. Both fields were mowed 3 d wk⁻¹ during active growth with a reel mower at 1.3 cm mowing height and clippings were returned. Irrigation was provided as needed to prevent turfgrass wilt. Annual bluegrass was actively growing and was at a multi-tiller growth stage on the day of initial treatments in both years. Annual bluegrass ground cover was visually evaluated on the day of initial treatment and was 20% (± 2) in 2013 and 45% (± 1) in 2014.

Treatments were the factorial arrangement of four herbicide/rate combinations and two application timings. Amicarbazone (Xonerate, 70WDG, Arysta LifeScience, Suite 150 Cary, NC 27513) was applied at 98 g ai ha⁻¹ followed by (fb) 98 g ha⁻¹ at 3 week after treatment (WAT), 196 g ha⁻¹ applied singly, or 392 g ha⁻¹ applied singly. Pronamide (Kerb 50WP, Dow Agrosciences LLC, 9330 Zionsville Rd, Indianapolis, IN 46268) at 1680 g ha⁻¹ was applied as a standard comparison. These treatments were applied at two seashore paspalum growth stages, including complete dormancy and transitional spring growth ($\approx 50\%$ greenup). Initial treatments for the two application timings were made on February 20 and March 13 in 2013 and February 24 and April 7 in 2014 for complete dormancy and 50% greenup, respectively. Applications were made with a CO₂ pressured sprayer calibrated to deliver 374 L ha⁻¹ with a single 9504E flat-fan nozzle (Tee Jet Spraying Systems Co., Roswell, GA 30075). Plots received ≈ 0.6 cm of irrigation at 24 hours after treatment.

Experimental design was a randomized complete block with four replications of 1 x 3-m plots. Annual bluegrass control was visually evaluated on a percent scale where 0 equaled no control and 100 equaled complete control. Turf injury was rated on a percent scale where 0

equaled no turf injury and 100 equaled complete desiccation. Data were subjected to analysis of variance with the General Linear Model procedure in SAS (SAS v. 9.3; SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513) at the 0.05 probability level. Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$. Year-by-treatment interactions were not detected for turfgrass injury, and thus results were pooled over years. Year-by-treatment interactions were significant for annual bluegrass control, and thus results are presented separately by year.

Summer experiments. Field experiments were conducted at the University of Georgia in Griffin, GA from July to August in 2011 and 2012. Soil was a Cecil sandy loam (Fine, kaolinitic, thermic Typic Kanhapluduts) with 2.5% organic matter and a pH of 6.2 on both fields. Plots used in 2012 were adjacent to plots in 2011. 'Tifway' bermudagrass and 'Sea Isle 1' seashore paspalum fairways were mowed 2 d wk⁻¹ with a reel-mower at a 1.6-cm height with clippings returned. Turfgrasses were irrigated as needed to prevent moisture stress.

Amicarbazone was applied to actively growing 'Tifway' bermudagrass and 'Sea Isle 1' seashore paspalum fairways at 0, 123, 246, 492, 738, or 984 g ha⁻¹. Spray solutions included a nonionic surfactant (Chem Nut 80-20, Chem Nut Inc., Albany, GA 31706) at 0.25% v/v. Treatments were applied using a CO₂-pressured sprayer calibrated to deliver 374 L ha⁻¹ with a single 9504E flat-fan nozzle. Application dates were July 5 in 2011 and July 2 in 2012.

Experiments were established as randomized complete block designs on both fields with four replications of 1 x 3-m plots. Bermudagrass and seashore paspalum injury was visually evaluated at 1, 2, and 4 WAT on a percent scale where 0 equaled no injury and 100 equaled completely dead turf. Turfgrass clippings were harvested at 2 and 4 WAT by making one pass down the center of each plot with a walk-behind reel-mower (Greensmaster 1000, Toro,

Minneapolis, MN 55401). Clippings were harvested on both fields at 3 d after the previous mowing, oven-dried at 60 C for 48 h, and weighed. Clipping yield reduction was calculated relative to the nontreated plot by replication on both fields. Yield of the nontreated plots are presented in Figure 2.

Data were subjected to analysis of variance in SAS. Turf injury and clipping reduction were plotted with amicarbazone rate and the following regression analysis was fit to the raw data, $y = a*[1-\exp(-b*x)]$. Standard error values and 95% confidence intervals were determined across all replications with SigmaPlot (Systat Software, Inc. 1735 Technology Drive, Suite 430 San Jose, CA 95110). Dose required to cause 20% visual injury (I_{20}) and clipping reduction (CR_{20}) were calculated from regression curves. For data presentation, means of each treatment are presented with standard errors in figures. Year-by-treatment interactions were not detected, and thus, results were pooled over years. Since species were grown on different fields, the results were analyzed as two separate experiments.

Results and Discussion

Winter and Spring Experiments. Seashore paspalum injury from amicarbazone and pronamide was minimal (<5%) from both application timings at weekly ratings throughout the experiments (data not shown). Moreover, no herbicide rate or timing reduced seashore paspalum spring greenup from the nontreated. In previous reports, seashore paspalum exhibited acceptable injury (<20%) from pronamide at 840 to 3360 g ha⁻¹ when applied at dormancy, 50% greenup, or 100% greenup (McCullough et al. 2012). Results suggest that seashore paspalum tolerance to amicarbazone is comparable to pronamide from winter and spring treatments.

Seashore paspalum is often injured excessively from POST herbicides used for annual bluegrass control in warm-season turfgrasses. For example, Johnson and Duncan (2000) reported that ethofumesate plus flurprimidol ($1.7 + 0.8 \text{ kg ha}^{-1}$) applied in April caused 61 to 65% injury to seashore paspalum from 2 to 6 WAT. Unruh et al. (2006) reported ethofumesate at 3.4 kg ha^{-1} injured seashore paspalum 60% at 2 WAT. In greenhouse experiments, atrazine and trifloxysulfuron caused excessive injury ($>20\%$) to seashore paspalum by 4 WAT (McCullough et al. 2012). Amicarbazone appears to be a safer alternative to these chemistries for controlling annual bluegrass before or during spring greenup of seashore paspalum.

By 6 WAT in 2013, treatments applied to dormant seashore paspalum and 50% turf greenup provided 51 and 57% control of annual bluegrass, respectively (Table 4.1). In 2014, herbicide applications at turf dormancy controlled annual bluegrass 69% but treatments at 50% turf greenup improved control to 78%. Amicarbazone at 392 g ha^{-1} and pronamide at 1680 g ha^{-1} averaged 79% annual bluegrass control in 2013 (Table 4.1). Amicarbazone applied sequentially at 98 g ha^{-1} or singly at 196 g ha^{-1} provided 24 and 48% control, respectively. In 2014, amicarbazone at 196 and 392 g ha^{-1} controlled annual bluegrass 71 and 90%, respectively, while pronamide at 1680 g ha^{-1} provided 81% control of annual bluegrass at 6 WAT. Similar to the results of 2013, sequential amicarbazone applications at 98 g ha^{-1} provided poor ($<70\%$) control of annual bluegrass in 2014.

Similar levels of annual bluegrass control in spring from amicarbazone applications were reported in previous research. McCullough et al. (2010) noted amicarbazone applied sequentially at 300 g ha^{-1} in spring provided 74% annual bluegrass control by 6 WAT in Indiana and New Jersey. Recently, Elmore et al. (2013) reported that spring amicarbazone applications at 150 and 255 g ha^{-1} controlled annual bluegrass 78 and 96% by 8 WAT, respectively. Amicarbazone

phytotoxicity to cool-season turfgrasses is exacerbated with increased temperatures (McCullough et al. 2010) and may explain the greater levels of annual bluegrass control from treatments at 50% turf greenup compared to applications at turf dormancy in 2014. Further investigations are warranted to evaluate the potential of early winter applications of amicarbazone to control seedling annual bluegrass prior to maturity in spring.

Summer Experiments. Seashore paspalum injury from amicarbazone rates ranged from 14 to 46%, 10 to 49%, and 6 to 41% by 1, 2, and 4 WAT, respectively (Figure 4.1). From regression analysis, seashore paspalum I_{20} values measured 220, 290, and 510 g ha⁻¹ by 1, 2, and 4 WAT, respectively. Seashore paspalum clipping yield reductions from amicarbazone rates ranged 0 to 31% by 2 WAT and increased by 27 to 51% by 4 WAT (Figure 4.2). Seashore paspalum CR_{20} values measured 520 and <123 g ha⁻¹ by 2 and 4 WAT, respectively.

Bermudagrass injury from amicarbazone rates ranged 3 to 23% at 1 WAT and decreased to <11% at 2 and 4 WAT (Figure 1). From regression analysis, bermudagrass I_{20} values measured 710 g ha⁻¹ by 1 WAT and >984 g ha⁻¹ by 2 and 4 WAT (Figure 4.1). Bermudagrass CR_{20} values measured >984 g ha⁻¹ at 2 and 4 WAT (Figure 4.2). Bermudagrass tolerance to summer amicarbazone applications is consistent with previous observations in field experiments (Yu and McCullough 2013).

Although injury from high rates was excessive, practitioners may safely apply amicarbazone at labeled use rates to seashore paspalum during active growth in summer. However, treatments reduced seashore paspalum clippings from the nontreated for 4 wk and could be problematic if active growth is needed. For example, excessive clipping reductions may concern sod growers harvesting in treated areas that require actively growing turf to sell.

Growth inhibition from amicarbazone applications could also reduce seashore paspalum recovery from disease, such as dollar spot (*Sclerotinia homoeocarpa* F. T. Benn.), or turf competition with summer weeds. Results suggest that end-users should be cautious with high rates of amicarbazone on seashore paspalum in summer if growth inhibition is objectionable.

Implications for seashore paspalum managers. Overall, amicarbazone appears to be safe for seashore paspalum during dormancy, spring transition, and active summer growth.

Amicarbazone efficacy was inconsistent over years and sequential treatments or high rates (392 g ha⁻¹) may be necessary to control annual bluegrass. Amicarbazone may also inhibit shoot growth of seashore paspalum for up to one month after treatment. This growth inhibition may concern practitioners and could be more pronounced on seashore paspalum than a more tolerant species, such as bermudagrass. Further investigation is needed to evaluate application rates and regimens of amicarbazone on other seashore paspalum cultivars at various seasonal timings.

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Table 4.1. Annual bluegrass control following treatments of amicarbazone and pronamide at two timings in field experiments, 2013 and 2014, Griffin, GA.

Application timing ^b		Annual bluegrass control (6 WAT ^a)	
		2013	2014
		-----%-----	
Dormancy		51	69
50% Greeup		57	78
	LSD _{0.05}		2
Herbicide	Rate (g ai ha ⁻¹)		
amicarbazone	98 fb ^c 98	24	53
	196	48	71
	392	78	90
pronamide	1680	80	81
	LSD _{0.05}	21	4
Timing		NS ^d	*
Herbicide		*	*
Herbicide x Timing		NS	NS

^a WAT = week after treatment.

^b Applications were made at two timings relative to seashore paspalum growth stages, including complete dormancy and 50% greenup. Applications were made on February 20 and March 13 in 2013 and February 24 and April 7 in 2014 for complete dormancy and 50% greenup of seashore paspalum, respectively.

^c fb = followed by.

^d NS, not significant at 0.05 probability level. * significant at the 0.05 probability level.

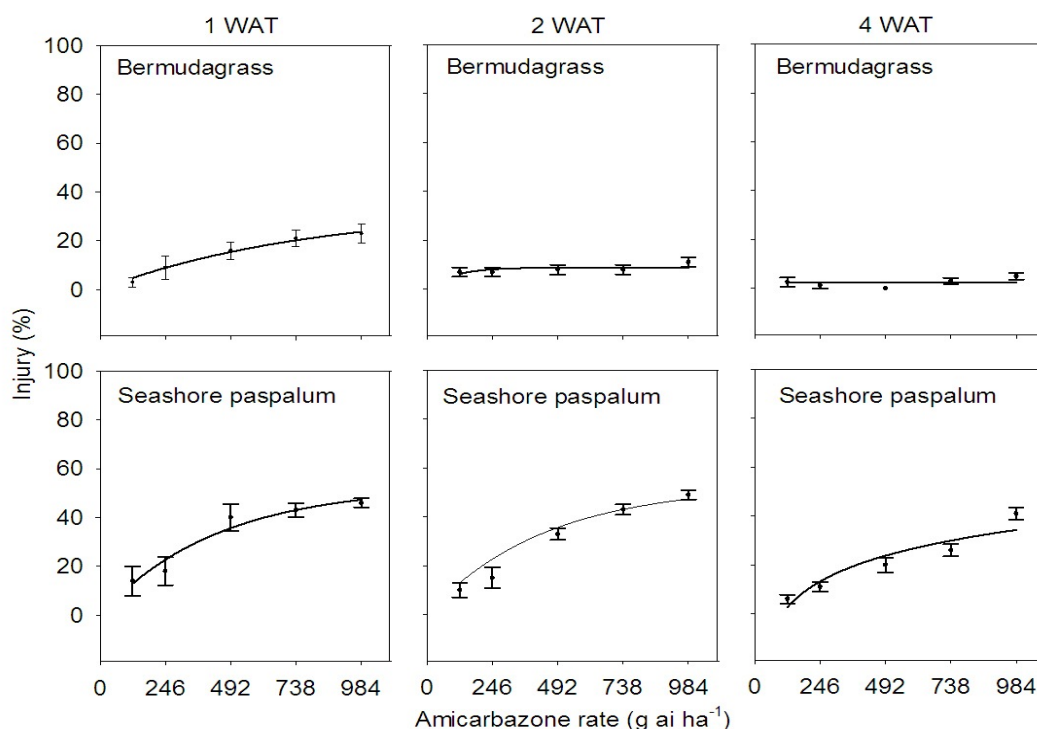


Figure 4.1. Percent bermudagrass and seashore paspalum injury after amicarbazone application in two combined field experiments, 2011-2012, Griffin, GA. Equations for 1 WAT: bermudagrass, $y = 32.31*(1-\exp(-0.0014*x))$, $r^2 = 0.38$, $SE = 9.67$, 95% CI = 17–24; seashore paspalum, $y = 53.36*(1-\exp(-0.0023*x))$, $r^2 = 0.51$, $SE = 13.12$, 95% CI = 16–29. Equations for 2 WAT: bermudagrass, NA; seashore paspalum, $y = 75.26*(1-\exp(-0.0011*x))$, $r^2 = 0.72$, $SE = 9.41$, 95% CI = 14–22. Equations for 4 WAT: bermudagrass, NA; seashore paspalum, $y = 1265.22*(1-\exp(-3.23E-5*x))$, $r^2 = 0.76$, $SE = 6.78$, 95% CI = 16–23. Vertical bars represent standard errors. Means represent the average of eight observations. Abbreviation: WAT = week after treatment, SE = standard error of estimate, 95% CI = 95% confidence interval.

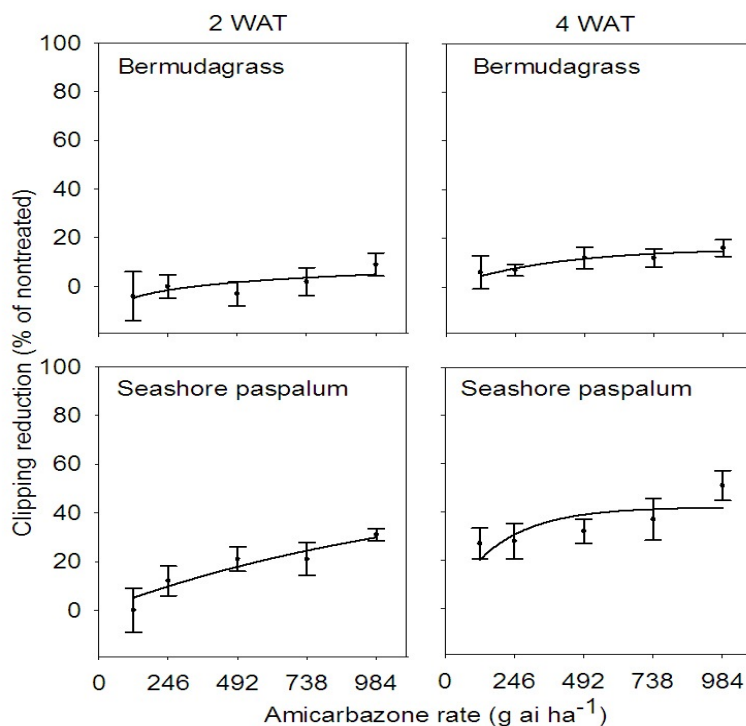


Figure 4.2. Percent bermudagrass and seashore paspalum clipping reductions after amicarbazone application in two combined field experiments, 2011-2012, Griffin, GA. Equations for 2 WAT: bermudagrass, NA; seashore paspalum, $y = 42.95*(1-\exp(-0.0012*x))$, $r^2 = 0.26$, SE = 17.62, 95% CI = 10–28. Equations for 4 WAT: bermudagrass, NA; seashore paspalum, $y = 45.54*(1-\exp(-0.0034*x))$, $r^2 = 0.20$, SE = 21.18, 95% CI = 6–24. Clipping weight (mean \pm standard error) of nontreated at 2 WAT: bermudagrass, $13 \pm 2 \text{ g m}^{-2}$; seashore paspalum, $10 \pm 1 \text{ g m}^{-2}$. Clipping weight (mean \pm standard error) of nontreated at 4 WAT: bermudagrass, $14 \pm 1 \text{ g m}^{-2}$; seashore paspalum, $10 \pm 1 \text{ g m}^{-2}$. Vertical bars represent standard errors. Means represent the average of eight observations. Abbreviation: WAT = week after treatment, SE = standard error of estimate, 95% CI = 95% confidence interval.

CHAPTER 5

OVERALL CONCLUSIONS

Annual bluegrass (*Poa annua* L.) is the most troublesome weed species in turfgrass. Currently, there are limited postemergence (POST) herbicides available for annual bluegrass control in cool-season turfgrasses because of inconsistent efficacy and turfgrass safety. Amicarbazone is a photosystem II inhibitor with potential to provide effective POST annual bluegrass control in cool-season turfgrasses and seashore paspalum.

Amicarbazone selectivity for annual bluegrass control in cool-season turfgrasses is associated with differential absorption, translocation, and metabolism. Annual bluegrass exhibited greater absorption and translocation of foliar and root applied ^{14}C -amicarbazone compared to creeping bentgrass (*Agrostis stolonifera* L.) and tall fescue (*Festuca arundinaceae* Shreb.). Additionally, annual bluegrass appears to have less ^{14}C -amicarbazone metabolism compared to creeping bentgrass and tall fescue. These responses contribute to the differential tolerance levels of phytotoxic effect of amicarbazone on these grasses.

Spring applications of amicarbazone effectively control annual bluegrass in bermudagrass (*Cynodon dactylon* (L.) Pers.) and tall fescue, but summer applications may excessively injure tall fescue. Greenhouse experiments suggest temperature has greater effect on turfgrass injury and shoot biomass reductions of annual bluegrass and tall fescue compared to bermudagrass following amicarbazone applications. Bermudagrass showed greater tolerance to amicarbazone treatments than tall fescue, despite exacerbated injury and shoot biomass reduction with increased temperatures. Tall fescue tolerance to amicarbazone decreased substantially as temperatures increased, and selectivity for annual bluegrass control is reduced at high

temperatures.

Amicarbazone foliar uptake in bermudagrass was comparable to tall fescue but less than annual bluegrass at low temperatures (25/20 °C). Foliar and root uptake of amicarbazone in annual bluegrass, bermudagrass, and tall fescue increased as temperature increased, but uptake in bermudagrass was less than annual bluegrass and tall fescue at high temperatures (40/35 °C). Averaged across species, grasses grown in higher temperatures distributed 10% more ¹⁴C to shoots compared to low temperatures. Metabolism of amicarbazone increased in annual bluegrass, bermudagrass, and tall fescue at high temperatures than low temperatures, but annual bluegrass had less metabolism than bermudagrass and tall fescue regardless of temperatures. The greater phytotoxic effects of amicarbazone on grasses at high temperatures than low temperatures are attributed to differential levels of herbicide absorption and translocation by roots and shoots of these grasses.

Amicarbazone was safe and effective for controlling annual bluegrass on bermudagrass and seashore paspalum in winter, spring, and summer at labeled use rates in field experiments. Amicarbazone applied sequentially at 98 g ha⁻¹, or singly at 196, and 392 g ha⁻¹ at dormancy or 50% turfgrass greenup caused minimal injury (5% or less) to seashore paspalum at weekly ratings throughout the experiments. No amicarbazone rates and timings reduced seashore paspalum spring greenup from the nontreated control. However, annual bluegrass control was inconsistent over years and high rates of amicarbazone at 392 g ha⁻¹ may be necessary to effectively control annual bluegrass.

Bermudagrass is relatively more tolerant to amicarbazone compared to seashore paspalum following the herbicide applications in summer. Summer amicarbazone applications may suppress seashore paspalum shoot growth for up to 4 weeks. This growth inhibition may

concern practitioners and could be more evident on seashore paspalum compared to bermudagrass.

In summary, amicarbazone selectivity for annual bluegrass control in cool-season turfgrasses is attributed to differential absorption, translocation, and metabolism. Physiological effects of temperature on annual bluegrass, bermudagrass, and tall fescue are attributed to differential levels of herbicide absorption and translocation by roots and shoots of these grasses. Moreover, amicarbazone is safe and effective for use in seashore paspalum during dormancy, spring transition, and active summer growth for controlling annual bluegrass and other weeds.