

ABSTRACT

JEFFRIES, MATTHEW DONNEL. Amicarbazone: Annual Bluegrass (*Poa annua* L.) Control and Creeping Bentgrass (*Agrostis stolonifera* L.) Tolerance. (Under the direction of Fred H. Yelverton).

Annual bluegrass (*Poa annua* L.) is the most troublesome weed in creeping bentgrass (*Agrostis stolonifera* L.) systems throughout the world. Previous reports confirm amicarbazone provides selective annual bluegrass control in cool-season turfgrass systems, including creeping bentgrass managed as golf course putting greens. However, further research is needed to provide optimal end-user application recommendations to manage annual bluegrass in this system. The objectives of this research were to evaluate annual bluegrass and creeping bentgrass growth responses to: 1) selective amicarbazone placement; and 2) various treatment regimens including amicarbazone and paclobutrazol.

Growth chamber experiments were conducted in 2010 and 2011 to determine the effects of soil-only, foliar-only, and soil + foliar amicarbazone placements on annual bluegrass and creeping bentgrass growth. Pooled over evaluated species, above-ground dry biomass reduction 56 days after treatment (DAT) was 26 and 64 % from foliar-only and soil-only placements, respectively. Further, root mass reduction 56 DAT followed similar trends, as foliar-only and soil-only placements caused 24 and 51 % reduction, respectively. No differences were observed for all evaluated parameters between soil-only and soil + foliar placements. Finally, annual bluegrass growth was consistently reduced more than creeping

bentgrass, confirming the potential for selective control of this weed species in creeping bentgrass systems.

Field trials were conducted throughout North Carolina in 2010 and 2011 to evaluate treatment regimens including amicarbazone (49, 65, or 92 g ai ha⁻¹) and the plant growth regulator, paclobutrazol (70, 140, or 280 g ai ha⁻¹). Treatment regimens included compounds applied as stand-alone treatments, tank-mixtures, or in tandem at varying rates and sequential timings. In general, regimens including amicarbazone applied at 49 or 92 g ha⁻¹ caused unacceptable turfgrass injury 8 weeks after initial treatment. Although three monthly applications of amicarbazone at 65 g ha⁻¹ did not provide > 41% annual bluegrass control throughout the course of this research, regimens including this application rate did provide acceptable turfgrass tolerance. Further, the addition of paclobutrazol increased control > 30% across all evaluated application rates. Based on this research, factors including amicarbazone placement, application rate, sequential timing, and paclobutrazol inputs should be taken into account when developing an annual bluegrass control program in creeping bentgrass systems.

Amicarbazone: Annual Bluegrass (*Poa annua* L.) Control and
Creeping Bentgrass (*Agrostis stolonifera* L.) Tolerance

by
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DEDICATION

This project is dedicated to my loved ones.

For it is the desire to make every one of you proud that has lead me down this road.

BIOGRAPHY

Matthew D. Jeffries was born July 27, 1987 in Huntsville, AL. At the age of 12 he relocated with his family to Asheboro, NC, where his parents still reside. As a youth, he played baseball and golf competitively for many years and during this time he routinely managed home lawns throughout the community. Upon entering the work force in high school, he found a natural fit as a golf course greenskeeper at Asheboro Country Club. It was there that he developed a genuine passion for turfgrass management. The desire to gain more knowledge in this field, coupled with a draw to anything outdoors lead him to pursue a B.S. degree in Turfgrass Science at North Carolina State University, which he completed with honors in 2009. Throughout his undergraduate career, he continued to work seasonally at Stoney Creek Golf Club in Burlington, NC and Desert Mountain Golf Properties in Scottsdale, AZ. Following the completion of his B.S. degree he interned as a field research assistant with BASF Corporation working in various crop systems. From this great opportunity rose another, as he began to work on a M.S. degree in Crop Science under the direction of Dr. Fred H. Yelverton. During the pursuit of his M.S. degree Matt focused his studies on turfgrass/weed management and soil/herbicide properties. It is his intention to apply this knowledge not only throughout the course of his career, but more specifically while working on a Ph. D. under the direction of Drs. Travis W. Gannon and Fred. H. Yelverton.

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A Review of the Literature

Annual Bluegrass

In many turfgrass systems throughout the United States (US) annual bluegrass (*Poa annua* L.) is considered a problematic weed. Annual bluegrass belongs to the *Poaceae* family, *Pooideae* subfamily, and *Poeae* tribe (Turgeon 2012). This weed species is an allotetraploid ($2n = 4x = 28$) originating from a cross between *Poa infirma* H. B. K., an annual, and *Poa supina* Schrad., a perennial (Beard 1973; Tutin 1952; Vargas and Turgeon 2004). Native to Europe, annual bluegrass is currently found throughout the world in subarctic, temperate, and subtropical climates (Beard 1973; Turgeon 2012). Within the US annual bluegrass is commonly found in cultivated areas, fields, pastures, turf, roadsides, railroad beds, and waste sites (Bryson and DeFelice 2009).

Annual bluegrass is widely considered a troublesome weed species due to copious seedhead production, poor heat and cold hardiness, as well as a high wilting tendency. Law et al. (1977) estimated annual bluegrass can produce 80 viable seeds per inflorescence when grown under low density. Inflorescence development has a detrimental impact on turfgrass aesthetics and functionality (Lush 1988a). Intolerance of extreme temperatures and wilting conditions also reduce turfgrass aesthetics and functionality, as areas previously infested with this weed are often left discolored or barren (McCarty 2001).

Despite the lifecycle suggested from the common name used throughout this review, annual bluegrass biotypes may demonstrate a perennial lifecycle in temperate and subarctic climates throughout the world (Turgeon 2012). The annual biotype, *Poa annua* L. var.

annua Timm., is as a bunch-type, non-creeping strain found where environmental stresses occur throughout the year, whereas the perennial biotype, *Poa annua* L.f. *reptans* (Houskins) Tokoyama, is a prostrate, creeping strain found under close mowing and frequent irrigation practices (Turgeon 2009; Warwick 1979). Inflorescence development characteristics vary between the two biotypes, with the former considered a prolific seedhead producer over a wide range of environmental conditions, and the latter demonstrating a more restricted, uniform onset of seedhead development in the spring, followed by vegetative growth through the summer (Beard 1970).

Due to frequent irrigation, fertilization, and fungicide applications, perennial biotypes of annual bluegrass are commonly found in creeping bentgrass (*Agrostis stolonifera* L.) putting greens throughout the US (Johnson and Murphy 1996). This scenario is especially problematic for chemical control, as bensulide, the only currently labeled PRE herbicide has virtually no activity on established perennial biotypes (Callahan and McDonald 1992). Field research evaluating paclobutrazol, a class B plant growth regulator used for annual bluegrass growth suppression, provides poor long term control (< 60 %) of this biotype as well (Johnson and Murphy 1996).

Annual bluegrass is commonly identified from vegetative characteristics including: folded vernation; membranous ligule 1-3 mm long; glabrous abaxial and adaxial leaf surfaces; and v-shaped leaf blades 2-3 mm wide with a boat-shaped tip (Beard 1973). Optimal temperature ranges for vegetative growth occur from 16 to 21 C (Beard 1970). Lush

(1989) reported plastochron development required 5.5 and 6.5 days leaf⁻¹ at constant temperatures of 22 and 32 C, respectively. When irrigated and maintained at an effective height of cut \leq 2.5 cm, annual bluegrass can form a dense, uniform turf of intermediate texture (Beard 1973; Lush 1988a). However, due to poor tolerance of stressful summer climatic conditions, this species is unable to provide a consistently acceptable turf surface over an extended period of time.

Depending on the biotype, annual bluegrass produces a fibrous to weakly stoloniferous root system (Mitich 1998). Research to date is inconclusive regarding potential rooting depth. This species has previously been described as shallow-rooted (Cooper et al. 1987; McCullough and Hart 2008); however, many claim this description is incorrect, as the shallow root systems observed were due to poor edaphic conditions (Beard 1970; Cordukes 1977; Vargas and Turgeon 2004). Excluding the first five months during establishment, Koski (1983) observed annual bluegrass rooting was consistently shallower over multiple years than creeping bentgrass, tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh.), perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.). Further, Karnok et al. (1982) reported creeping bentgrass root length was on average two- to three-fold greater than that of annual bluegrass from mid-April to mid-May in two consecutive years. Research conducted by Wilkinson and Duff (1972) counter the aforementioned conclusions regarding annual bluegrass rooting. The authors found no significant differences between annual bluegrass and creeping bentgrass root mass at varying soil bulk densities;

however, it was stated the soil texture (sandy loam) plants were grown in coupled with the maximum bulk density evaluated (1.4 g cm^{-3}) were inadequate to restrict root growth.

Optimal temperature ranges for annual bluegrass root growth occur from 10 to 16 C (Beard 1970). Annual bluegrass demonstrates a trimodal root growth pattern over the course of a year (Vargas and Turgeon 2004). This atypical growth pattern for turfgrasses is attributed to carbohydrate depletion caused by inflorescence development during the spring season, resulting in two root elongation periods in spring and one during fall (Danneberger and Vargas 1984; Vargas and Turgeon 2004). Ong and Marshall (1975) reported annual bluegrass allocated 55% of total plant assimilates to inflorescence development at 30 days after seedhead emergence; however, inflorescence removal redirected assimilates back to the roots, stems, and tillers.

Annual bluegrass produces a terminal panicle inflorescence composed of three- to eight-flowered spikelets (Warwick 1979). Inflorescence development is possible under routine mowing at an effective height of cut $\geq 3 \text{ mm}$ (Christians 2011; Turgeon et al. 2009). While inflorescence development is possible year-round, annual bluegrass seedhead production is greatest during spring (Lush 1988a). Danneberger and Vargas (1984) reported maximum flowering in the Midwest US occurred over a 14 – 18 d period beginning in mid-May to early June. Further, maximum annual bluegrass seed density ($210,000 \text{ seeds m}^{-2}$) in a golf course putting green was observed in the spring, with winter, summer, and fall densities reduced from the spring approximately 80, 45, and 80 %, respectively (Lush

1988b). Johnson and White (1997) hypothesized inflorescence development was catalyzed by a vernalization period. The researchers found four perennial biotypes all required a vernalization period of 10 to 12 wk at 4 and 8 C; however, all four biotypes responded variably to vernalization treatments in terms of time-to-flowering and number of inflorescences developed. Inflorescence development of the true annual bluegrass biotype evaluated was not dependent on a vernalization period.

Annual bluegrass inflorescence development occurs via self- or cross-pollination. Koshy (1969) reported the total number of germinated seed set per spikelet was enhanced by cross-pollination. Turgeon (2012) stated up to 5% crossing between different annual bluegrass populations is possible. This affinity for cross-pollination partially explains the wide genotypic and phenotypic variability demonstrated by this species in various turf systems throughout the world. Once germination occurs, short seed ripening periods give this weed the ability to quickly infest a recently disturbed area. Evaluations of nine populations revealed germination was possible (< 7%) when inflorescences were removed from the parent plant at anthesis, while germination rates of seed collected from all populations evaluated ranged from 5 – 26 % when inflorescences were removed four days following anthesis (Koshy 1969). Finally, Cattani et al. (2002) suggested annual bluegrass is able to outcompete many turfgrass species including creeping bentgrass at establishment due to a comparatively larger seed size, and inherently a greater endosperm storage capacity for plant growth in early stages (1 – 2 leaves). Turgeon et al. (2012) reported average annual bluegrass and creeping bentgrass seed mass to be 0.55 and 0.07 mg, respectively.

Dissemination of seed via wind, water, human activity, or animal consumption are the primary processes by which annual bluegrass is introduced to an area (Mitich 1998).

Previous reports regarding conditions necessary for annual bluegrass seed germination are highly variable. In general, germination occurs over a wide temperature range, with some reduction in very low or very high temperatures (Vargas and Turgeon 2004). McElroy et al. (2004) reported maximum germination ($\geq 81\%$) of eight annual bluegrass populations in a greenhouse setting at 19/10 C day/night environment. Reduced germination rates for all populations were observed with an increase in temperature to 29/19 C. Observations over multiple years in Maryland coincide with the previously reported data. Researchers found that most annual bluegrass germination (76%) occurred over a three to four week period beginning in late September. Mean daily temperatures during this time period generally were ≤ 20 C (Kaminski and Dernoeden 2007). Shem-Tov and Fennimore (2003) also reported germination rates were highest during a similar time period; however, the authors concluded that seed dormancy state, rather than temperature was responsible for the routine emergence patterns observed. Soil samples taken in March and November were incubated for 45 d under spring or fall conditions. Average daily maximum and minimum temperatures ranged < 3 C from the aforementioned sampling dates. The authors reported seedling emergence from soil samples taken in March did not germinate under either seasonal condition, whereas germination rates under both spring and fall conditions were $> 95\%$ from samples taken in November.

The dependence of annual bluegrass germination on varying environmental conditions during dormancy has been previously reported. Lush (1988a) reported germination percentages after 14 d under 25/15 C day/night conditions were 71%; however, germination increased to 91% if seeds were previously chilled at 5 C for 7 d. Annual bluegrass seed from this research was obtained from a regularly irrigated putting green near Melbourne, Australia. Seed collected from a comparatively warmer climate in south Louisiana demonstrated a vastly different response to temperature during dormancy, as germination of dormant dried and imbibed seeds increased with increasing storage temperatures from 10 – 30 C (Standifer and Wilson 1988a). It was proposed this high temperature requirement was an adaptation to the subtropical climate found in south Louisiana. Further research from Standifer and Wilson (1988b) showed annual bluegrass populations from varying climates in the US demonstrate variable expressions of seed dormancy. Seeds collected from Louisiana, Maryland, and Wisconsin were subjected to constant temperatures of 5, 10, 15, and 20 C. Seed collected from Wisconsin germinated > 40% at all evaluated temperatures, whereas Maryland seed germination peaked at 10 C (80%) and then sharply declined to 30 and 0 % germination at 15 and 20 C, respectively. The Louisiana population did not germinate at any temperature. The authors concluded annual bluegrass grows as a winter annual in the relatively warmer climates of Louisiana and Maryland, but as a summer annual in Wisconsin due to the less stressful summer climatic conditions. Finally, dormancy characteristics may vary widely within a given area due to the conditions parent plants developed under. Lush (1989) described annual bluegrass

populations from a golf course rough, fairway, and putting green near Melbourne, Australia. The author reported populations from the fairway and rough were similar to each other but differed from the putting green population in many attributes including germination. Seeds collected from the putting green germinated readily, whereas seeds from the other areas on the golf course required a chilling (5 C) treatment. It was speculated the favorable growth conditions associated with greens management coupled voids in the turf canopy left from deceased parent plants provided the conditions necessary for rapid germination, whereas seed in areas less intensively managed required a chilling period to avoid stressful summer climatic conditions.

Affects of Creeping Bentgrass Management on Annual Bluegrass Growth

Annual bluegrass is the most problematic weed in creeping bentgrass putting greens (Turgeon et al. 2009). Beard et al. (1978) stated a cultural program for controlling this weed should be designed to optimize the competitive ability of the specific turfgrass cultivar in use. Considerations include: (a) reasonable use of the turf; (b) the timing, amount, and form of plant nutrient application through fertilization; (c) the amount, frequency, and uniform distribution of water provided through irrigation; (d) the height, frequency, pattern, and method of mowing; (e) and the method, timing, and severity of cultivation. The scope of this portion of the review is not to discuss every cultural practice for optimal creeping bentgrass putting green management; however, the aforementioned considerations will be addressed with regard to their potential impact(s) on annual bluegrass growth.

To date, creeping bentgrass is the most widely used cool-season turfgrass for golf course putting greens in the transition zone and cool humid regions of the US (Liu and Huang 2001; Turgeon 2012). Native to Eurasia, creeping bentgrass forms an extremely fine textured, dense playing surface when managed appropriately (Beard 1973). Creeping bentgrass is best adapted to fertile, fine textured soils of moderate acidity (pH 5.5 – 6.5) and good water holding capacity; however, this species demonstrates poor growth characteristics when subjected to compacted soils (Beard 1973).

Creeping bentgrass is described as a shallow to moderately rooted species, with rooting depths peaking in the spring (Beard 1973; Vargas and Turgeon 2004). Growth chamber experiments conducted by Beard and Daniel (1965) revealed similar root growth rate from 16 – 21 C, but was reduced considerably at 32 C. Field research conducted by Liu and Huang (2001) support the aforementioned findings, as turf quality, canopy net photosynthesis rate, and leaf photochemical efficiency were highest in May and June of two consecutive years, and lowest in July through September. Differing tolerances to stressful climatic conditions were observed between evaluated varieties, with turf quality estimations highest for ‘L-93’, lowest for ‘Penncross’, and intermediate for ‘Providence’ and ‘Crenshaw’. Decline in turf quality and rooting is commonly observed on creeping bentgrass putting greens when daily air temperatures are routinely > 30 C (Carrow 1996; Fry and Huang 2004). This is due to high photorespiration rates, which decreases photoassimilate allocation for root growth (Turgeon 2012). Considerations regarding summer creeping bentgrass decline are important for annual bluegrass management due to the potential for

reduced turfgrass vigor and coverage in late summer and early fall, leaving an ideal set of conditions for annual bluegrass germination and establishment. Finally, annual bluegrass establishment in putting greens is impacted by the variety of creeping bentgrass present. After transplanting annual bluegrass plugs into monostands of thirteen creeping bentgrass varieties, Beard et al. (2001) found that varieties with shoot densities $\geq 2000 \text{ dm}^{-2}$ were better suited to reduce annual bluegrass establishment pressure than less dense varieties. Overall, annual bluegrass was more persistent over time in plots containing eight traditional, coarser-textured varieties (37% average cover) compared to four recently developed, finer-textured varieties (8% average cover).

Few variations occur when comparing nutritional requirements of annual bluegrass and creeping bentgrass; however, differing growth responses between species associated with iron, nitrogen, and phosphorus applications will be briefly discussed. Xu and Mancino (2001) reported creeping bentgrass and annual bluegrass shoot dry mass increased 70 and 0 %, respectively, as iron application rate increased from 0 to 6 ppm. Root growth increased with iron concentration similarly across both species. Timely iron applications during annual bluegrass germination periods may hinder this process due to resource depletion associated with vigorous creeping bentgrass vegetative growth. Creeping bentgrass has a lower optimal nitrogen requirement than annual bluegrass, therefore judicious applications of this nutrient should be exercised. Further, split-applications of nitrogen should be made at lower rates when both species are actively growing in a vegetative state (Vargas and Turgeon 2004). When annual bluegrass seedhead development initiates in the spring, increased nitrogen

application rates can be utilized due to the reduced ability of annual bluegrass to respond vegetatively (Vargas and Turgeon 2004). Annual bluegrass growth responses following phosphorus applications are not only due to the essentiality of this nutrient for plant growth, but are also due to the impact phosphorus has on soil pH. Varco and Sartain (1966) reported annual bluegrass clipping yield from various concentrations of $\text{Ca}(\text{OH})_2$ was increased as phosphorus rate increased. This interaction was attributed to annual bluegrasses high requirement of phosphorus as a plant nutrient, an increase in soil pH from added phosphorus, and less Al^{3+} uptake due to aluminum-phosphorus, adsorption-precipitation reactions at the root surface. Further, with adequate N-P-K- levels, Juska and Hanson (1969) reported an increase in soil pH from 4.5 to 6.5 doubled annual bluegrass foliage mass and quadrupled seedhead counts. Finally, creeping bentgrass demonstrates a competitive advantage over annual bluegrass regarding phosphorus uptake in acidic soil conditions. Greenhouse experiments evaluating phosphorus uptake at pH 4.7 revealed creeping bentgrass tissue phosphorus concentration and total phosphorus uptake were 17 and 32 % higher, respectively, than annual bluegrass (Kuo et al. 1992).

Regardless of playing surface quality expectations, all creeping bentgrass putting greens in the transition zone and southwest US require routine irrigation to survive stressful environmental conditions (Dunn and Diesberg 2004). Fu and Dernoeden (2009) reported supplying irrigation at leaf wilt to saturate the soil to a 24 cm depth promoted favorable root growth compared to irrigating daily to a 6 cm depth. Similar rooting trends were observed by Madison (1961) on highlands bentgrass (*Agrostis tenuis* Sibth.) and seaside bentgrass

(*Agrostis palustris* Huds.). The researcher reported root mass at the 0 - 15 cm depth increased by 35% when plots were irrigated weekly compared to delivering an equivalent volume of water in five irrigation cycles throughout the week (Madison 1961). Irrigation scheduling considerations are not only important to promote creeping bentgrass root growth, but also have a substantial impact on annual bluegrass control. Gaussoin (1989) concluded annual bluegrass survivability and germinability increased with irrigation frequency. Although the author did not provide an explanation for this occurrence, the increased survivability is likely due to higher soil moisture content at or near the surface where annual bluegrass rooting is commonly restricted within due to poor edaphic conditions (Cooper et al. 1987; Vargas and Turgeon 2004). Annual bluegrass germination was enhanced likely for the same reason. Further supporting this theory, Allen et al. (1993) determined annual bluegrass seed viability was not impacted by various hydration/dehydration cycles; however, annual bluegrass germination occurred more rapidly as dehydration phase length decreased from 24 to 0 h.

The increased emphasis placed on creeping bentgrass putting green quality and ball roll distances over time has caused golf course superintendents to compromise fundamental cultural practices, namely by mowing at, or below recommended heights (≈ 3.5 mm) (Beard et al. 2001; McCullough et al. 2005a). Previous research has shown reducing height of cut from 4.8 to 3.2 mm can cause nearly a three-fold decrease in subsurface (7.5 – 15 cm) creeping bentgrass root densities (Salaiz et al. 1995). Krans and Beard (1985) concluded the decrease in root growth with reduced mowing heights is due to reduced leaf surface area and

consequently, a reduced photosynthetic capacity per plant. Liu and Huang (2003) reported a reduction from 4 to 3 mm in the mowing height of ‘Crenshaw’ and ‘Penncross’ creeping bentgrass decreased turf quality, net photosynthesis rate, and leaf photochemical efficiency, whereas respiration rate and soil temperatures increased. This is concerning, as a weakened turf stand coupled with increased soil temperatures could enhance annual bluegrass germination and establishment. Although clippings are typically removed from a putting green surface following a mowing event due to playability issues, Gaussoin (1989) found this practice also reduces annual bluegrass populations over time. The author reported annual bluegrass coverage decreased 12% over three years when clippings were not returned to the putting green. Further, seed banks were reduced > 50% in two consecutive years when clippings were not returned.

The perennial nature and intensive management of creeping bentgrass putting greens inevitably causes an increase in soil organic matter and thatch-mat depth over time (McCarty et al. 2005; Turgeon 2012). To reduce soil organic matter accumulation, cultural practices such as aerification and vertical mowing are commonly utilized (Fu et al. 2009; McCarty et al. 2005). Although these practices are necessary to provide a quality playing surface over the lifetime of a putting green, their disruptive nature on the turf canopy and soil profile provide an opportunity for annual bluegrass germination and establishment (Lush 1988b). Younger (1959) reported light renovation of dormant bermudagrass (*Cynodon dactylon* L.) increased annual bluegrass population counts approximately nine times compared to non-disrupted areas. The author attributed this population shift to an increased

amount of light, moisture, and oxygen available for germination and establishment. As stated previously, annual bluegrass is a prolific producer of readily germinable seed in putting green systems (Lush 1988b; Turgeon 2009). Therefore, the timing of these disruptive practices should coincide with optimal creeping bentgrass vegetative growth, minimizing the window of time for voids in the turf canopy.

Chemical Annual Bluegrass Control in Creeping Bentgrass Putting Greens

There are currently no effective herbicide options for annual bluegrass in creeping bentgrass putting greens (Turgeon et al. 2009). PRE herbicide options are limited, as bensulide is the only currently labeled compound for use in creeping bentgrass putting greens. Hart et al. (2004) reported applications of bensulide at 11.2 kg ai ha⁻¹ in September did not decrease 'L-93' creeping bentgrass coverage or root mass from the nontreated. Further, excellent *Poa annua* L. var. *annua* control (97%) has been documented with bensulide at the previously mentioned rate followed by two sequential applications at 6 kg ha⁻¹; however, maximum control of *Poa annua* L.f. *reptans* was < 18% over four years of field research (Callahan and McDonald 1992). To date, there are no registered POST herbicides for annual bluegrass control in creeping bentgrass putting greens. Bispyribac-sodium and ethofumesate are available for POST annual bluegrass control in creeping bentgrass maintained at, or above a golf course tee and fairway mowing height, respectively; however, these compounds are not registered for use at lower mowing heights due to the potential for unacceptable turf injury (Lewis and DiPaola 1989; McCullough and Hart 2009; Teuton et al. 2007).

Plant growth regulators (PGRs) inhibiting gibberellin-biosynthesis are the best options currently available for annual bluegrass control in creeping bentgrass putting greens (Turgeon et al. 2009; Vargas and Turgeon 2004). Paclobutrazol, a class B PGR, suppresses annual bluegrass growth more than creeping bentgrass (Koski 1997). Beneficial impacts on paclobutrazol treated plants include reduced vegetative biomass, plant/internode length, inflorescence development and water consumption, as well as increased chlorophyll content and tolerance to extreme temperatures (Gilley and Fletcher 1997; Navarro et al. 2007; Pinhero and Fletcher 1994). Further, improved creeping bentgrass quality from increased shoot density and dark green color has been documented following applications of paclobutrazol. Koski (1997) noted over 58 weekly ratings, turf quality and density were higher in treated plots on 38 and 35 rating dates, respectively. Short-term (7 – 10 d) phytotoxic effects were observed 7 d after application. McCullough et al. (2005b) reported spring applications of paclobutrazol at 0.56 kg ai ha⁻¹ followed by 0.28 kg ha⁻¹ three weeks later caused ≥ 53% annual bluegrass coverage reduction in two consecutive years. Similar results were obtained from four biweekly paclobutrazol applications at 0.11 kg ha⁻¹ initiated mid-September followed by five applications beginning in March. Averaged over two years of research, Bell et al. (2004) reported this treatment regime caused 82% annual bluegrass coverage reduction. Trends in creeping bentgrass quality paralleled Koski's findings. To date, evaluations of paclobutrazol for *Poa annua* L.f. *reptans* control reveal the need for other chemical options. Johnson and Murphy (1996) reported three paclobutrazol applications in the spring and fall totaling 2.6 kg ha⁻¹ suppressed the perennial biotype 80%

relative to the nontreated 3 weeks after the final treatment; however, suppression declined 23% over the following 13 weeks. The authors concluded multiple applications over an indefinite time period would be required to suppress annual bluegrass populations to acceptable levels.

Recent research suggests amicarbazone (4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide), a photosystem II (PSII) inhibiting herbicide, may have utility for annual bluegrass control in creeping bentgrass putting greens. Reports have confirmed this herbicide provides excellent control (> 90%) of annual bluegrass; however, unacceptable injury has been documented on creeping bentgrass putting greens (McCullough et al. 2010; Yelverton 2009; Yelverton 2010). McCullough et al. (2010) reported amicarbazone applications at 0.3 kg ai ha⁻¹ on ‘L-93’ creeping bentgrass in mid-October caused 37 and 8 % injury in Indiana and New Jersey, respectively. When applications were delayed to April, injury was reduced at both locations to 11 and 0 %, respectively, four weeks after treatment. Amicarbazone applications in mid-April and early May at 0.3 kg ha⁻¹ provided 74% annual bluegrass control at both locations six weeks after the initial treatment. Yelverton (2009) also found amicarbazone applied in the fall caused unacceptable creeping bentgrass injury, as amicarbazone applied at 0.26 kg ha⁻¹ caused > 95% injury when applied in September and 0% injury when applied the following April. Further research found four weekly applications of amicarbazone at 0.049 kg ha⁻¹ beginning in late March caused ≤ 11% creeping bentgrass injury and > 80% annual bluegrass control (Yelverton 2010). Finally, McCullough et al. (2010) reported annual bluegrass and ‘L-93’

creeping bentgrass injury increased with increasing temperature from 10 to 30 C across all application rates evaluated (0.1, 0.2, 0.3, 0.4, and 0.5 kg ha⁻¹), therefore, the usage of this herbicide on creeping bentgrass putting greens should be limited to the spring.

Photosystem II Inhibition

Photosystem II is a protein complex within the thylakoid membrane of chloroplasts. In the presence of light, energy stored in excited chlorophyll molecules is used for photolysis, yielding protons, electrons, and free oxygen (O₂) (Lawlor 2001). Electrons are transported within the complex to the chlorophyll p680⁺ reaction center. Within the D1 protein, an oxidized tyrosine residue donates an electron to the reaction center forming p680[·]. The reduced reaction center donates its electron to phaeophytin, a chlorophyll molecule lacking magnesium. Phaeophytin then transports an electron to the stationary electron acceptor, plastoquinone A (PQ_A) on the D2 protein. Finally, electrons are directed to the mobile two-electron carrier, PQ_B on the D1 protein. Once fully reduced, this protein-bound quinone is protonated, forming plastohydroquinone (PQH_{2B}). The binding affinity of this quinone species is poor, and is displaced by PQ_B (Cobb and Reade 2010). Electrons are subsequently transported by PQH_{2B} to the cytochrome *b₆f*-complex, which supplies electrons for PSI (Oettemeier 1999).

Photosystem II-inhibiting herbicides have superior binding kinetics to plastoquinone at the PQ_B-binding site on the D1 protein. Once herbicides outcompete for these sites, electron flow is halted and singlet energy state chlorophyll molecules accumulate (Hess

2000). This accumulation leads to the formation of triplet energy state chlorophyll. Although triplet chlorophyll is naturally produced in photosynthesis, susceptible plants are not able to process the abundance of this chlorophyll species before free oxygen radicals (primarily singlet oxygen [O₂*] and hydrogen peroxide [H₂O₂]) are formed (Hess 2000). These reactive oxygen species cause lipid peroxidation, destroying cell membrane integrity and eventually causing plant death (Hess 2000). Symptoms from PS II-inhibitors slowly develop over several days. Initially, treated plants develop a marked chlorosis, which is followed by necrosis. The chlorosis is due to chlorophyll destruction through photo-oxidation reactions in the chloroplast, and the necrosis is due to membrane destruction through lipid peroxidation (Turgeon 2009).

Amicarbazone

Amicarbazone is a carbamoyl triazolinone herbicide that controls susceptible plants by inhibiting PSII (Senseman 2007). Triazolinone herbicides share a three nitrogen triazole ring with an oxygen doubly-bonded to one of the ring's carbons (Turgeon 2009). Other triazolinone herbicides commonly used in turf include carfentrazone and sulfentrazone.

Originally registered for broadleaf weed control in corn (*Zea mays* L.; PRE and PPI) and sugarcane (*Saccharum officinarum* L.; PRE and POST), amicarbazone is currently being evaluated for weed control in turf systems (Dayan et al. 2009; Senseman 2007). Research has found amicarbazone applied at 0.25 to 0.50 kg a.i. ha⁻¹ effectively controls velvetleaf (*Abutilon theophrasti* Medik.), common lambsquarters (*Chenopodium album* L.), common

cocklebur (*Xanthium strumarium* L.), pigweed species (*Amaranthus* spp.), and morningglory species (*Ipomoea* spp.) (Kramer et al. 2012; Senseman 2007). Susceptible plants demonstrate the following symptoms: chlorosis, stunted growth, tissue necrosis beginning at leaf edges and progressing inward, and eventual death (Senseman 2007).

Physicochemical properties pertinent to amicarbazone environmental fate include: no dissociation in slightly acidic to slightly basic soils, high water solubility (4,600 ppm at 20 C), low vapor pressure (9.75×10^{-9} mm Hg at 30 C), a moderate affinity for soil sorption ($\log K_{ow} = 1.23$ at pH 7 and 20 C), and a short to moderate half-life in aerobic soils ($T_{1/2} = 19 - 87$ days under field conditions) (US EPA 2005). These properties suggest amicarbazone is susceptible to lateral transport across the soil surface during periods of heavy rainfall/irrigation or downward movement through the soil profile as moisture approaches saturation (Anonymous 2005). Field studies conducted by Bachege et al. (2009) aimed to determine the leaching potential of amicarbazone and sulfentrazone. After a 106 mm accumulated rainfall, sulfentrazone and amicarbazone leached 10 and > 35 cm, respectively. These findings confirm amicarbazone is a highly soil-mobile compound and applications of this herbicide should not be made when lateral movement across, or downward movement through the soil profile is likely. Conditions favoring lateral movement include applications made on fine-textured soils, whereas downward movement is more likely when amicarbazone is applied to coarse-textured soils. Off-target movement in both scenarios is exacerbated when an application is made to saturated soils, or if excessive

irrigation/precipitation inputs are introduced to the system within a 24 h period following an application.

Amicarbazone uptake occurs via root and foliar pathways; however, Negrisoli et al. (2007) reported weed control was highest when the herbicide was applied directly to the soil or immediately leached from herbicide-treated sugarcane straw, suggesting root uptake is an impactful route for plant growth inhibition from amicarbazone applications (Dayan et al. 2009). Differential plant uptake associated with herbicide placement has been previously documented for quinclorac and bispyribac-sodium on torpedograss (*Panicum repens* L.) and annual bluegrass, respectively (Lycan and Hart 2006; Williams et al. 2004). In both cases weed control was enhanced when the herbicides were applied directly to the soil surface. Finally, Wehtje et al. (1997) reported control of yellow nutsedge (*Cyperus esculentus* L.) and purple nutsedge (*Cyperus rotundus* L.) was enhanced when sulfentrazone, a fellow triazolinone herbicide, was applied selectively to the soil surface in addition to only plant foliage. The authors found no significant differences in final tuber mass between foliage-only sulfentrazone (140 – 280 g ai ha⁻¹) and the control; however, differences from the control were observed on all species and rates evaluated from soil + foliage placements. Aside from the need to determine the utility of amicarbazone for weed control in turf systems, a better understanding of the effect of amicarbazone placement on plant growth is also necessary to implement this herbicide in an efficient and environmentally-friendly manor.

Objectives

Amicarbazone has shown potential utility for selective annual bluegrass control in creeping bentgrass putting greens. Further research is required to successfully integrate this herbicide into turf management programs. The objectives of this research were to investigate:

1. the effect of amicarbazone placement on annual bluegrass and creeping bentgrass growth.
2. spring treatment regimens including amicarbazone and paclobutrazol for annual bluegrass control in creeping bentgrass putting greens.

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Chapter 1: Effects of Postemergence Amicarbazone Placement on Annual Bluegrass (*Poa annua* L.) and Creeping Bentgrass (*Agrostis stolonifera* L.) Growth.

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Growth chamber experiments were conducted to assess the impacts of soil-only, foliar-only, and soil + foliar placements of amicarbazone on annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.) growth. Evaluated herbicide treatments included amicarbazone applied at 49 or 147 g ai ha⁻¹, as well as bispyribac-sodium at 74 g ai ha⁻¹ for comparative purposes. Pooled over plant species, both soil placements of amicarbazone applied at 49 g ha⁻¹ more than doubled above-ground biomass reduction 56 days after treatment (DAT), compared to foliar-only placement. Pooled over herbicide placement, annual bluegrass and creeping bentgrass above-ground biomass was reduced 64 and 29 %, respectively, from amicarbazone at the aforementioned rate, confirming the potential for selective annual bluegrass control in creeping bentgrass systems. Root mass reduction 56 DAT followed similar trends across both amicarbazone application rates. Across all evaluated parameters in this research, amicarbazone applied at 49 g ha⁻¹ impacted creeping bentgrass growth similarly to bispyribac-sodium, while annual bluegrass growth was inhibited more by the latter herbicide, suggesting it provides a more efficacious chemical option for end-user applications. Data from this research coincide with previous reports of

amicarbazone uptake, as this compound is sorbed via above- and below-ground pathways; however, plant growth is inhibited most by root uptake.

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Nomenclature: amicarbazone; bispyribac-sodium; creeping bentgrass, *Agrostis stolonifera* L.; annual bluegrass, *Poa annua* L. var. *annua*

Key words: Foliar absorption, root absorption, herbicide placement, herbicide efficacy, digital image analysis.

Introduction

Annual bluegrass is a problematic cool-season weed that negatively impacts turfgrass aesthetics and functionality in warm- and cool-season turfgrass systems throughout the world. Indigenous to Europe, this weed is predominately propagated by seed and spread through human interaction (Beard 1970; Gaussoin 1990). When compared to other properly managed turfgrass species, annual bluegrass is a lighter shade of green and is capable of producing abundant seed heads at golf course putting green mowing heights (≈ 3 mm), leaving a mottled appearance and uneven playing surface (Christians 2011; Lush 1988). Further, annual bluegrass demonstrates poor heat hardiness and drought tolerance during summer conditions common to the transition, warm arid, and warm climatic zones (Beard 1970). Turf quality and playing surface quality are often decreased following stressful climatic conditions in areas infested with annual bluegrass due to turf discoloration or coverage reduction (McCarty 2001).

Creeping bentgrass is the most widely used cool-season turfgrass species on golf course putting greens in the United States due to its fine texture, prostrate growth habit, and tolerance to low mowing heights (> 3 mm) (Salaiz et al. 1995; Turgeon 1980). Native to Eurasia, creeping bentgrass is commonly used for all playing surfaces in cool-humid regions, but is predominately utilized as a putting green surface in warmer regions of the US (Beard 1973). Although this species does provide a high quality playing surface, creeping bentgrass has inherent difficulty growing in late summer months due to intensive cultural management coupled with stressful environmental conditions, resulting in a weakened

turfgrass stand prone to pest infestations (Beard and Daniel 1966; Carrow 1996). This makes creeping bentgrass putting greens susceptible to annual bluegrass invasion, as this weed germinates predominately in early fall (Kaminski and Dernoeden 2007; Shem-Tov and Fennimore 2003).

Regardless of the setting, annual bluegrass is the most troublesome weed in creeping bentgrass systems (Beard 1973; Turgeon 2012). To date, POST herbicide control options are limited (Turgeon 2012). Woosley et al. (2003) reported three ethofumesate applications ($0.85 \text{ kg ai ha}^{-1}$) on a creeping bentgrass fairway provided 62% annual bluegrass coverage reduction 10 wk after final treatment (WAFT); however, annual bluegrass coverage was equivalent to nontreated plots 36 WAFT. The authors reported the addition of paclobutrazol, an early-stage gibberellin biosynthesis inhibitor, was necessary to obtain significant annual bluegrass control one year following treatment regimen application initiation (Kwon and Yim 1986; Woosley et al. 2003). Further, ethofumesate is not currently registered for usage on putting greens due to potential unacceptable turfgrass injury (Anonymous 2012; Lewis and DiPaola 1989). Bispyribac-sodium, an acetolactate synthase inhibitor, is used for POST annual bluegrass control in creeping bentgrass systems as well; however, this herbicide is also not registered on creeping bentgrass at putting green mowing heights due to potential unacceptable turfgrass injury (McCullough and Hart 2009; Teuton et al. 2007). Further, Lycan and Hart (2006b) reported creeping bentgrass injury was exacerbated as temperatures declined from 15 C shortly after bispyribac-sodium

applications, narrowing the application window to late spring and early summer when annual bluegrass is more mature and difficult to control (Rao 2000).

In 2012, amicarbazone received registry in the US for annual bluegrass control in creeping bentgrass systems, including golf course roughs, fairways, and tees (Anonymous 2012). Recent research suggests amicarbazone also has utility for annual bluegrass control in creeping bentgrass putting greens (Warren et al. 2009; Yelverton 2009). Amicarbazone is a photosystem II inhibiting herbicide within the triazolinone family (Senseman 2007).

Symptoms produced on susceptible plants include chlorosis, stunted growth, tissue necrosis beginning at the leaf edges and progressing across and downward over leaf and stem tissue, and eventual death (Senseman 2007). Amicarbazone is currently registered in South Africa for PRE and POST applications in sugarcane (*Saccharum officinarum* L.) and in Brazil for PPI and POST applications in corn (*Zea mays* L.) (Anonymous 2011; Dayan et al. 2009; Dewar 2003). Research has found amicarbazone applied at 0.25 to 0.50 kg ai ha⁻¹ effectively controls velvetleaf (*Abutilon theophrasti* Medik.), common lambsquarters (*Chenopodium album* L.), common cocklebur (*Xanthium strumarium* L.), pigweed species (*Amaranthus* spp.), and morningglory species (*Ipomoea* spp.) (Kramer et al. 2012; Senseman 2007).

Herbicide placement has been found to be important in the control of various grass and sedge species. When comparing quinclorac and bispyribac-sodium applied only to plant foliage or the soil surface, the soil placement more effectively inhibited torpedograss (*Panicum repens* L.) and annual bluegrass growth, respectively (Williams et al. 2004; Lycan and Hart 2006a). Dayan et al. (2009) found amicarbazone was readily absorbed in foliage

and roots of large crabgrass (*Digitaria sanguinalis* (L.) Scop.). Although a foliar application for comparison was not included in the research, Negrisoli et al. (2007) reported weed control was highest when amicarbazone was applied directly to the soil or immediately leached from herbicide-treated sugarcane straw with 30 mm rainfall (the maximum water volume evaluated), which indicated herbicide sorption via root uptake. Finally, Wehtje et al. (1997) reported control of yellow nutsedge (*Cyperus esculentus* L.) and purple nutsedge (*Cyperus rotundus* L.) was enhanced when sulfentrazone, a fellow triazolinone herbicide, was applied to the soil surface in addition to the foliage. The authors found no significant differences in final tuber mass between foliar-only sulfentrazone (0.14 – 0.28 kg ai ha⁻¹) and the control; however, differences from the control were observed on all species and rates evaluated from soil + foliar placements.

To date, information pertaining to the influence of selective amicarbazone placement on annual bluegrass and creeping bentgrass growth is limited. This information is essential for developing effective application methods and post-application irrigation scheduling. The objective of this research was to determine the effect of POST amicarbazone placement on annual bluegrass and creeping bentgrass growth.

Materials and Methods

Trials were conducted in 2010 and 2011 at the Southeastern Plant Environment Laboratory at North Carolina State University, Raleigh, NC, USA to investigate the effect of soil-only, foliar-only, and soil + foliar application placements of amicarbazone and bispyribac-sodium on annual bluegrass and ‘A1’ creeping bentgrass growth. Growth

chamber temperature conditions were 24/13 (± 1) C day/night, with a 14 h day-length period. At plant level, the measured photosynthetically active radiation flux was $555 \mu\text{mol m}^{-2} \text{s}^{-1}$. Locally collected annual bluegrass seed (Thorndale Country Club, Oxford, NC) and purchased 'A1' creeping bentgrass seed (Tee-2-Green Corporation, Hubbard, OR) was sown into plastic pots (182 cm^2 surface area, 1670 cm^3 volume) filled with a sand-based growth medium containing 2.6% organic matter at pH 6.2. A surplus of pots was established to allow selection of uniform plants at experiment initiation. Plants were irrigated by hand twice daily, fertilized weekly with a 20-20-20 water soluble-fertilizer (Peters Professional 20-20-20 Water Soluble Fertilizer, Scotts-Sierra Horticultural Products Company, Marysville, OH) to provide $1.2 \text{ g N-P-K m}^{-2}$, and clipped weekly to a 3.75 cm height. Fertilizer applications were not made 2 wk before or after trial initiation. Following a 90 d maturation period, at trial initiation annual bluegrass plants averaged 10 – 14 tillers and creeping bentgrass plants covered 33 cm^2 of the soil surface.

Experiments were conducted as a randomized complete block design with a two by three by three factorial treatment arrangement and four replications. Factorial levels include two grass species (annual bluegrass or creeping bentgrass), three herbicide treatments (amicarbazone [Amicarbazone 70 WG[®], Arysta LifeScience Corporation, Cary, NC] at 49 or 147 g ai ha^{-1} , or bispyribac-sodium [Velocity 17.6 SG[®], Valent U.S.A. Corporation, Walnut Creek, CA] at 74 g ai ha^{-1} , and three herbicide placements (foliar-only, soil-only, or foliar + soil). A nontreated check was included in each replicate and pots were re-randomized weekly to minimize chamber effects. Amicarbazone treatments included a non-ionic

surfactant (Induce[®], Helena Chemical Company, Collierville, TN) at 0.25% vol vol⁻¹. Foliar-only and foliar + soil treatments were applied with a hand-held CO₂-pressurized sprayer calibrated to deliver 304 L ha⁻¹ with one 8002E flat fan nozzle (TeeJet[®] flat-fan nozzles, Spraying Systems Co., Wheaton IL) at 262 kPa. Herbicide to soil contact was inhibited for foliar-only treatments by placing a 2.5 cm layer of activated charcoal (BL Powdered Activated Carbon[®], Calgon Carbon Corporation, Pittsburgh, PA) on the soil surface prior to treatment applications. The activated charcoal barrier was removed 24 h after treatments were applied. Soil-only treatments were prepared by diluting the appropriate amount of active ingredient that would contact the pot soil surface for a given herbicide application rate in 10 ml tap water. Herbicide solutions were then uniformly syringed over the soil surface, preventing herbicide to foliage contact. Pots were not irrigated 24 h prior to and 48 h following experiment initiation. Irrigation was syringed every other day over the soil surface to replenish soil water content to approximately 90% soil saturation for 14 days after treatment (DAT) to prevent displacing herbicide from plant foliage or leaching through the soil profile. Soil saturation was determined for each pot before trials were initiated on a mass basis. This was determined by subtracting the mass of a given pot at saturation with the average mass of eight unused pots not irrigated for a 14 d period. Pots were weighed every other day and the difference between 90% soil saturation and the observed saturation state was converted to a water volume. After this time period, irrigation methods resumed. Finally, grass foliage was not clipped for 28 d following trial initiation.

Digital image analysis (DIA) was taken 14, 28, 42, and 56 DAT to determine percent green foliage coverage within an experimental unit using SigmaScan Pro (SigmaScan Pro[®], version 5, Systat Software Incorporated, Chicago, IL) as previously described (Karcher and Richardson 2005; Richardson et al. 2001). Images were captured utilizing a digital camera (Canon PowerShot SD750[®], Canon Incorporated, Lake Success, NY) mounted to a tripod (Sony VCT-R649 Lightweight Tripod[®], Sony Corporation of America, New York, NY) with the tripod neck fully extended 90° from vertical, 51 cm directly above each pot. Green foliage coverage over a given pot was calculated and percent green foliage cover reduction relative to the nontreated within a replicate was calculated by:

$$[1] \% \text{ cover} = [((C_{NT} - C_T) / C_{NT}) \times 100]$$

where C_T and C_{NT} equaled coverage in treated and nontreated pots, respectively. Plants were clipped to a 2.5 cm height 28 DAT and harvested mass was recorded after clippings were dried for 14 d at 70 C. All plants were allowed to re-grow, then destructively sampled 56 DAT. At that time, above-ground biomass was harvested at the soil surface and roots were washed free of soil using a showerhead hose nozzle and 1.4 mm sieve. Samples were dried under the same conditions as previously described for clippings. After the 14 d drying period, above-ground biomass was recorded and root mass was then determined through loss by ignition (Koshi 1997). Samples were ashed in a muffle furnace at 500 C for 12 h and root mass was then determined by subtracting the initial sample mass from the residual mineral mass. Percent clipping mass, above-ground biomass and root mass reduction were calculated identically to DIA data.

After confirming homogeneity of variance, data were subject to ANOVA ($P = 0.05$). Plant species, herbicide treatment, herbicide placement, and experimental run were considered fixed effects (Table 1). Main effects and their interactions are presented accordingly, with precedent given to interactions of increasing magnitude (Steele et al., 1997). Means were separated according to Fisher's protected LSD ($P < 0.05$) with the use of SAS general linear models (Statistical Analysis Software[®], version 9.2, SAS Institute Incorporated, Cary, NC).

Results and Discussion

Plant Biomass Reduction

A species by herbicide placement interaction on clipping mass reduction data was detected 28 DAT. Pooled over herbicide treatment, foliar-only and soil-only herbicide placements on annual bluegrass clipping mass were additive, as foliar-only, soil-only, and foliar + soil placements caused 16, 47, and 64 % reduction, respectively (Table 2). No differences were observed among placement levels within creeping bentgrass; however, all placement levels caused 31 – 45 % clipping mass reduction. This reduction in vegetative growth is concerning for turf managers due to reduced creeping bentgrass recuperative ability. Further, actively growing turf is necessary for optimal root production in the spring season when amicarbazone and bispyribac-sodium applications are recommended. Impacts from reduced vegetative growth in the spring season may result in decreased heat and drought stress tolerance during stressful summer conditions in the following months.

Significant plant species by herbicide treatment, and herbicide treatment by herbicide placement interactions on above-ground biomass reduction were detected 56 DAT. Pooled over herbicide placement, amicarbazone at 49 and 147 g ha⁻¹ responded similarly, causing 64 and 71 % annual bluegrass biomass reduction, respectively, whereas bispyribac-sodium caused 31% reduction (Table 3). Creeping bentgrass biomass reduction followed similar trends, with amicarbazone at 49 and 147 g ha⁻¹ causing 29 and 42 % reduction, respectively, and bispyribac-sodium a 15% reduction. Once again, no differences were detected between amicarbazone application rates. Although direct comparisons between species cannot be made, the bispyribac-sodium response agrees with previous research suggesting the compound can selectively inhibit annual bluegrass growth in creeping bentgrass systems (Lycan and Hart 2006b; Teuton et al. 2007). Pooled over plant species, above-ground biomass reduction 56 DAT was dependent on soil exposure for both amicarbazone application rates, as no differences were detected between soil-only and soil + foliar placements (Table 3). Foliar-only placement of amicarbazone at 49 and 147 g ha⁻¹ reduced biomass 25 and 27 %, respectively, whereas soil-only and soil + foliar placements caused 59 and 72 % reduction, respectively, averaged over soil placements. Bispyribac-sodium responded similarly across all placement levels.

Plant species by herbicide treatment, and herbicide treatment by herbicide placement interactions were detected on root mass reduction data 56 DAT. Pooled over herbicide treatment, no differences were detected between soil-only and soil + foliar placements for either species evaluated (Table 4). Foliar-only placement caused 35 and 13 % root mass

reduction of annual bluegrass and creeping bentgrass, respectively. Averaged over soil placements, annual bluegrass and creeping bentgrass root mass were reduced 65 and 40 %, respectively. Pooled over plant species, no differences were detected between soil-only and soil + foliar placements for both evaluated amicarbazone application rates (Table 4). Foliar-only placement of amicarbazone at 49 and 147 g ha⁻¹ caused 30 and 23 % root mass reduction, respectively. Averaged over soil placements, root mass reduction from amicarbazone at 49 and 147 g ha⁻¹ more than doubled foliar-only placement, causing 62 and 73 % reduction, respectively. Bispyribac-sodium responded similarly across all placement levels, ranging from 19 – 27 % root mass reduction. Contrary to this data, Lycan and Hart (2006a) reported soil and foliar + soil placements of bispyribac-sodium caused more annual bluegrass and creeping bentgrass shoot growth reduction than foliar applications, 28 DAT. Bispyribac-sodium application rate for the presented research was ¼ and ½ the rates used by the authors. Further, discrepancies between results may be explained by our use of more mature plants at trial initiation, a greater potential rooting volume, and later root mass assessments following trial initiation.

Green Foliage Cover Reduction

Due to a plant species by herbicide treatment by herbicide placement interaction detected on green foliage coverage reduction 28 and 56 DAT, data were separated by plant species. For each data collection date, the effect of plant species and herbicide placement

within an herbicide treatment is presented first, followed by the effect of herbicide treatment and herbicide placement within a plant species.

Annual bluegrass and creeping bentgrass responded similarly to foliar-only placement of amicarbazone at 49 g ha^{-1} 28 DAT, causing 12 and 6 % green foliage coverage reduction, respectively (Table 5). Plant species responded differently to soil-only and foliar + soil placements at the aforementioned rate. Soil-only placement on annual bluegrass and creeping bentgrass caused 49 and 7 % coverage reduction, respectively, while foliar + soil placement caused 58 and 7 % coverage reduction, respectively. Differences in coverage reduction were detected between annual bluegrass and creeping bentgrass to amicarbazone at 147 g ha^{-1} at all placement levels. Foliar-only, soil-only, and foliar + soil placements caused 16, 63, and 68 % annual bluegrass coverage reduction, whereas, foliar-only, soil-only, and foliar + soil placements caused 4, 8, and 7 % creeping bentgrass coverage reduction, respectively. Annual bluegrass and creeping bentgrass responded differently to foliar-only and foliar + soil placements of bispyribac-sodium. Annual bluegrass coverage was reduced 30 and 37 % from foliar-only and foliar + soil placements, respectively, whereas creeping bentgrass coverage was reduced 7 and 6 %, respectively.

Within annual bluegrass, herbicide placement impacted green foliage coverage reduction from amicarbazone at both application rates 28 DAT. In general, annual bluegrass green foliage coverage was reduced more by both soil placements than the foliar-only placement. Soil-only and foliar + soil placements of amicarbazone at 49 g ha^{-1} caused 49 and 58 % green foliage cover reduction, whereas the foliar-only placement caused 12% coverage

reduction (Table 5). Similarly, soil-only and foliar + soil placements of amicarbazone at 147 g ha⁻¹ caused 63 and 68 % coverage reduction, respectively, whereas the foliar placement caused 16% coverage reduction. No differences were observed between soil-only and foliar + soil placements at either amicarbazone application rate. Within creeping bentgrass, no differences were detected between herbicide treatment and herbicide placement on green foliage coverage reduction 28 DAT. Creeping bentgrass coverage was reduced $\leq 8\%$ at all treatment levels.

Annual bluegrass and creeping bentgrass responded differently to all placement levels of amicarbazone applied at 49 and 147 g ha⁻¹ 56 DAT. Foliar-only, soil-only, and foliar + soil placements of amicarbazone at 49 g ha⁻¹ caused 32, 74, and 76 % annual bluegrass green foliage cover reduction, respectively, whereas creeping bentgrass coverage was reduced -0.8, 2.3, and 6.5 % from foliar-only, soil-only, and foliar + soil placements, respectively (Table 6). Negative coverage reduction values denote an increase in coverage, relative to the nontreated. Foliar-only, soil-only, and foliar + soil placements of amicarbazone at 147 g ha⁻¹ caused 18, 83, and 83 % annual bluegrass cover reduction, respectively, whereas creeping bentgrass coverage was reduced -1.5, 0.5, and 2.3 % from foliar-only, soil-only, and foliar + soil placements, respectively. Varying responses between species also occurred with foliar-only and foliar + soil placements of bispyribac-sodium. Annual bluegrass green foliage coverage was reduced 34 and 27 % from foliar-only and foliar + soil placements, respectively, whereas creeping bentgrass coverage was slightly increased 1.6 and 0.9 % relative to the nontreated from foliar-only and foliar + soil placements, respectively.

Within annual bluegrass, herbicide placement had a significant impact on green foliage coverage reduction for amicarbazone at both application rates 56 DAT. In general, annual bluegrass green foliage coverage was reduced more by both soil placements than the foliar-only placement. Soil-only and foliar + soil placements of amicarbazone at 49 g ha⁻¹ caused 74 and 76 % coverage reduction, respectively, whereas foliar-only placement caused 32% reduction (Table 6). Similarly, soil-only and foliar + soil placements of amicarbazone at 147 g ha⁻¹ caused 83 and 83 % coverage reduction, respectively, whereas foliar-only placement caused 18% coverage reduction. Within creeping bentgrass, slight differences in green foliage cover reduction were observed between herbicide treatment and placement 56 DAT; however all treatments reduced green foliage < 7%, an acceptable trade-off by turf managers for annual bluegrass control (Table 6).

Based on our results, amicarbazone plant uptake is influenced by herbicide placement. Although plant absorption is possible via foliar- and root-uptake as previously reported (Dayan et al. 2009), amicarbazone uptake had greater impact on plant growth when applied to the soil surface. Negrisoni et al. (2007) reported similar results, as weed control was highest when amicarbazone was applied directly to the soil surface or displaced by rainfall from amicarbazone-treated straw. Research including POST sulfentrazone applications, an herbicide in the same chemical family as amicarbazone, provided similar results, as acceptable control of purple nutsedge (*Cyperus rotundus* L.) and yellow nutsedge (*Cyperus esculentus* L.) was only obtained when sulfentrazone was allowed to contact the soil surface, demonstrating the importance of root absorption of this compound (Wehtje et al.

1997). Across herbicide placements, annual bluegrass was more susceptible to amicarbazone than creeping bentgrass. Across species and amicarbazone application rates, soil-only and soil + foliar placements provided comparable growth reduction, whereas the foliar-only placement had less impact. When comparing soil-only and foliar + soil placements of bispyribac-sodium and amicarbazone, the latter herbicide clearly provides more promise for POST annual bluegrass control from this research; however, injury to our desirable turfgrass from soil placement of amicarbazone is a point of concern. For this reason, turfgrass managers should ensure creeping bentgrass systems are well-established before initiating a root-directed amicarbazone application to reduce potential unacceptable injury. Future research should address methods to promote herbicide-soil contact including cultural practices to increase soil exposure and post-application irrigation scheduling.

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Table 1. Analysis of variance for annual bluegrass and creeping bentgrass clipping mass reduction, above-ground biomass reduction, root mass reduction, and green foliage cover reduction.^{a, b, c}

| Source of variation | df | % reduction | | | | |
|---------------------|----|--------------------------------------|----------------------|---------------------|-----------------------|-----------------------|
| | | 28 DAT ^d clipping mass | 56 DAT AG-biomass | 56 DAT root mass | 28 DAT green cover | 56 DAT green cover |
| Run (R) | 1 | NS | NS | NS | NS | NS |
| Species (S) | 1 | NS | *** ^e | *** | *** | *** |
| Herbicide (H) | 2 | NS | *** | *** | *** | *** |
| Placement (P) | 2 | *** | *** | *** | *** | *** |
| R x S | 1 | NS | NS | NS | ** | NS |
| R x H | 2 | NS | NS | NS | NS | NS |
| R x P | 2 | NS | NS | NS | NS | NS |
| S x H | 2 | NS | * | NS | *** | *** |
| S x P | 2 | * | NS | * | *** | *** |
| H x P | 4 | NS | *** | * | *** | *** |
| R x S x H | 2 | NS | NS | NS | NS | NS |
| R x S x P | 2 | NS | NS | NS | NS | NS |
| R x H x P | 4 | NS | NS | NS | NS | NS |
| S x H x P | 4 | NS | NS | NS | *** | *** |
| R x S x H x P | 4 | NS | NS | NS | NS | NS |

^a Percent mass reduction and coverage reduction data are relative to the nontreated check within a given replicate.

^b All mass reduction data is based on dried samples.

^c Green coverage measured using SigmaScan[®] software on a 0 – 100 % scale, based on 0 = no green foliage and 100 = complete green foliage coverage.

^d Abbreviations: DAT, days after treatment; AG, above-ground; NS, not significant at P = 0.05 level.

^e Significance at P < 0.05, P < 0.01, and P < 0.001 levels denoted by *, **, and ***, respectively.

Table 2. Species by herbicide placement interaction on clipping mass reduction, 28 DAT.^{a, b, c}

| Placement | POAAN | AGSST |
|---------------------|-------|-------|
| | % | |
| Foliage | 16 | 31 |
| Soil | 47 | 31 |
| Foliage + soil | 64 | 45 |
| LSD _{0.05} | 16 | NS |

^a Percent oven-dried clipping mass reduction, relative to the nontreated.

^b Data pooled over herbicide treatments.

^c Abbreviations: DAT, days after treatment; POAAN, annual bluegrass; AGSST, creeping bentgrass; NS, not significant at P = 0.05 level.

Table 3. Species by herbicide treatment interaction and herbicide treatment by herbicide placement interaction on above-ground biomass reduction, 56 DAT.^{a, b}

| Herbicide ^c | g ha ⁻¹ | % | | % | | | LSD ^d |
|------------------------|--------------------|-------|-------|---------|------|----------------|------------------|
| | | POAAN | AGSST | Foliage | Soil | Foliage + soil | |
| Amicarbazone | 49 | 64 | 29 | 25 | 56 | 62 | 14 |
| Amicarbazone | 147 | 71 | 42 | 27 | 72 | 72 | 10 |
| Bispyribac | 74 | 31 | 15 | 27 | 16 | 28 | NS |
| LSD ^e | | 9 | 14 | | | | |

^a Abbreviations: DAT, days after treatment; POAAN, annual bluegrass; AGSST, creeping bentgrass; NS, not significant at P = 0.05 level.

^b Percent oven-dried above-ground biomass reduction, relative to the nontreated.

^c All amicarbazone treatments applied with a non-ionic surfactant at 0.25% vol vol⁻¹.

^d LSD (P < 0.05) for comparing herbicide placement and herbicide treatment, pooled over species.

^e LSD (P < 0.05) for comparing species and herbicide treatment, pooled over herbicide placement.

Table 4. Species by herbicide placement interaction and herbicide treatment by herbicide placement interaction on root mass reduction, 56 DAT.^{a, b, c}

| Placement | POAAN | AGSST | Amicarb. (49) ^{d, e} | Amicarb. (147) | Bispyribac (74) |
|---------------------|-------|-------|-------------------------------|----------------|-----------------|
| | % | | % | | |
| Foliar | 35 | 13 | 30 | 23 | 19 |
| Soil | 62 | 40 | 63 | 72 | 18 |
| Foliar + soil | 67 | 40 | 60 | 73 | 27 |
| LSD _{0.05} | 10 | 8 | 12 | 7 | NS |

^a Abbreviations: DAT, days after treatment; POAAN, annual bluegrass; AGSST, creeping bentgrass; Amicarb, amicarbazone; NS, not significant at P = 0.05 level.

^b Root mass determined by loss through ignition.

^c Percent root mass reduction, relative to the nontreated.

^d Herbicide treatment application rate: g ha⁻¹.

^e All amicarbazone treatments applied with a non-ionic surfactant at 0.25% vol vol⁻¹.

Table 5. Effect of herbicide treatment and herbicide placement on annual bluegrass and creeping bentgrass green foliage cover reduction, 28 DAT.^{a, b, c}

| Herbicide ^d | g ha ⁻¹ | | POAAN | | AGSST | LSD ^e |
|------------------------|--------------------|---------------|-------|----|-------|------------------|
| | | | % | | | |
| Amicarbazone | 49 | Foliar | 12 | 6 | } | 15 |
| | - | Soil | 49 | 7 | | |
| | - | Foliar + soil | 58 | 7 | | |
| Amicarbazone | 147 | Foliar | 16 | 4 | } | 10 |
| | - | Soil | 63 | 8 | | |
| | - | Foliar + soil | 68 | 7 | | |
| Bispyribac | 74 | Foliar | 30 | 7 | } | 20 |
| | - | Soil | 12 | 8 | | |
| | - | Foliar + soil | 37 | 6 | | |
| LSD ^f | | | 19 | NS | | |

^a Abbreviations: DAT, days after treatment; POAAN, annual bluegrass; AGSST, creeping bentgrass; NS, not significant at P = 0.05 level.

^b Percent green foliage cover reduction, relative to the nontreated.

^c Green foliage coverage measured using SigmaScan[®] software on a 0 – 100 % scale, based on 0 = no green foliage and 100 = complete green foliage coverage.

^d All amicarbazone treatments applied with a non-ionic surfactant at 0.25% vol vol⁻¹.

^e LSD (P < 0.05) for comparing herbicide placement and species within a herbicide treatment.

^f LSD (P < 0.05) for comparing herbicide treatment and herbicide placement within a species.

Table 6. Effect of herbicide treatment and herbicide placement on annual bluegrass and creeping bentgrass green foliage cover reduction, 56 DAT.^{a, b, c, d}

| Herbicide ^e | g ha ⁻¹ | | POAAN | | AGSST | LSD ^f |
|------------------------|--------------------|---------------|-------|------|-------|------------------|
| | | | % | | | |
| Amicarbazone | 49 | Foliar | 32 | -0.8 | } | 11 |
| | - | Soil | 74 | 2.3 | | |
| | - | Foliar + soil | 76 | 6.5 | | |
| Amicarbazone | 147 | Foliar | 18 | -1.5 | } | 10 |
| | - | Soil | 83 | 0.5 | | |
| | - | Foliar + soil | 83 | 2.3 | | |
| Bispyribac | 74 | Foliar | 34 | -1.6 | } | 20 |
| | - | Soil | 12 | 0.1 | | |
| | - | Foliar + soil | 27 | -0.9 | | |
| LSD ^g | | | 20 | 3.6 | | |

^a Abbreviations: DAT, days after treatment; POAAN, annual bluegrass; AGSST, creeping bentgrass; NS, not significant at P = 0.05 level.

^b Percent green foliage cover reduction, relative to the nontreated.

^c Green foliage coverage measured using SigmaScan[®] software on a 0 – 100 % scale, based on 0 = no green foliage and 100 = complete green foliage coverage.

^d Negative green foliage cover reduction values indicate an increase in green foliage cover, relative to the nontreated.

^e All amicarbazone treatments applied with a non-ionic surfactant at 0.25% vol vol⁻¹.

^f LSD (P < 0.05) for comparing herbicide placement and species within a herbicide treatment.

^g LSD (P < 0.05) for comparing herbicide treatment and herbicide placement within a species.

Chapter 2: Amicarbazone and Paclobutrazol for Annual Bluegrass (*Poa annua* L.) Control on Creeping Bentgrass (*Agrostis stolonifera* L.) Putting Greens.

Formatted for Weed Technology

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Amicarbazone is a photosystem II-inhibiting herbicide recently registered for annual bluegrass control in cool-season turf systems, including creeping bentgrass. However, research to date reveals potential issues with creeping bentgrass tolerance, as seasonal and climatic conditions greatly impact amicarbazone turf tolerance. The plant growth regulator paclobutrazol has been widely adopted by turf managers for annual bluegrass control on creeping bentgrass putting greens. Field experiments were conducted throughout North Carolina in the spring seasons of 2010 and 2011 to assess the efficacy of amicarbazone (49, 65, or 92 g ai ha⁻¹) and paclobutrazol (70, 140, or 280 g ai ha⁻¹) for annual bluegrass control in creeping bentgrass putting greens. The herbicides were applied alone, as tank-mixtures, or used in tandem at varying rates and sequential timings. In general, regimens including both compounds provided greater annual bluegrass control and acceptable creeping bentgrass tolerance compared to stand-alone applications of amicarbazone 8 and 12 weeks after initial treatment (WAIT). When comparing regimens including amicarbazone at 49 or 65 g ai ha⁻¹, creeping bentgrass tolerance was greater for the latter application rate 8 WAIT. These results indicate amicarbazone usage on creeping bentgrass greens may be beneficially impacted with

the incorporation of paclobutrazol to the treatment program, as annual bluegrass control was equivalent or superior to stand-alone amicarbazone applications, and creeping bentgrass tolerance was superior.

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Nomenclature: amicarbazone; paclobutrazol; annual bluegrass, *Poa annua* L.; creeping bentgrass, *Agrostis stolonifera* L., 'A1', 'A4', 'Crenshaw', and 'L-93'.

Key words: annual bluegrass control, digital image analysis, injury, quality, turf spectral reflectance.

Introduction

Annual bluegrass is the most troublesome weed in creeping bentgrass systems throughout the United States (US) (Turgeon et al. 2009). Native to Europe, annual bluegrass is widely considered a troublesome weed species due to copious seedhead production, poor heat tolerance and cold hardiness, and a high wilting tendency (Beard 1970). Law et al. (1977) estimated annual bluegrass can produce 80 viable seeds per inflorescence when grown under low density. Further, annual bluegrass has the ability to produce seedheads at golf course putting green mowing heights (≈ 3 mm) (Christians 2011; Turgeon et al. 2009). Inflorescence development has a detrimental impact on turfgrass playability, often interfering with ball roll on putting greens (Lush 1988). Intolerance of extreme temperatures and wilting conditions also reduce turfgrass aesthetics and functionality, as areas previously infested with this weed are often left discolored or barren (McCarty 2001).

Creeping bentgrass is the most widely used cool-season turfgrass species for golf course putting greens in the transition zone and cool humid regions of the US (Liu and Huang 2001; Turgeon 2012). Native to Eurasia, creeping bentgrass forms an extremely fine textured, dense playing surface (Beard 1973). Creeping bentgrass is best adapted to fertile, fine textured soils of moderate acidity (pH 5.5 – 6.5) and good water holding capacity; however, this species demonstrates poor growth characteristics when subjected to compacted soils (Beard 1973). Although this turf species provides a high quality playing surface, creeping bentgrass has inherent difficulty surviving in late summer months under intensive cultural management practices coupled with unfavorable environmental conditions common

in the southern boundaries of its distribution, resulting in a weakened turfgrass stand prone to pest infestations (Carrow 1996).

Currently, there are no POST herbicides available for annual bluegrass control in creeping bentgrass putting greens (Turgeon et al. 2009). Bispyribac-sodium and ethofumesate are available for POST annual bluegrass control in creeping bentgrass at, or above a 12.5 mm height of cut; however, unacceptable turf injury can result from these herbicides at putting green mowing heights (Lewis and DiPaola 1989; McCullough and Hart 2009; Teuton et al. 2007). The most widely utilized chemical option currently registered for annual bluegrass suppression in creeping bentgrass putting greens is paclobutrazol, a class B plant growth regulator (PGR) that inhibits early stage gibberellin-biosynthesis (Turgeon et al. 2009; Vargas and Turgeon 2004). Koski (1997) concluded annual bluegrass control from paclobutrazol applications on creeping bentgrass putting greens is due to differential growth suppression, as growth of the former species is suppressed more so than the latter. Bell et al. (2004) reported four bi-weekly paclobutrazol applications at 110 g ai ha⁻¹ initiated mid-September, followed by five applications beginning in March, reduced annual bluegrass coverage in early April 79 and 85 % relative to the nontreated in 2002 and 2003, respectively. Aside from providing annual bluegrass control, improved overall creeping bentgrass quality from increased shoot density and darker green color has been documented following paclobutrazol applications (Koski 1997; Woosley et al. 2003). Koski (1997) noted over 58 consecutive weekly ratings, creeping bentgrass quality and density were higher in paclobutrazol treated plots on 38 and 35 rating dates, respectively. To date, evaluations of

paclobutrazol for control of *Poa annua* L.f. *reptans*, the perennial biotype of annual bluegrass commonly found on putting greens, reveals the need for other chemical options. Johnson and Murphy (1996) reported three paclobutrazol applications in the spring and fall totaling 2.6 kg ha⁻¹ suppressed the perennial biotype 80% relative to the nontreated 3 weeks after the final treatment; however, suppression declined 23% over the following 13 weeks. The authors concluded multiple applications over an indefinite amount of years are required to suppress populations of this annual bluegrass biotype to acceptable levels.

In 2012, amicarbazone received registry in the US for annual bluegrass control in creeping bentgrass systems, including golf course roughs, fairways, and tees (Anonymous 2012). Amicarbazone is a photosystem II-inhibiting herbicide belonging to the triazolinone herbicide family (Senseman 2007). Although amicarbazone is registered for PRE sugarcane (*Saccharum officinarum* L.) applications in South Africa and PPI corn (*Zea mays* L.) applications in Brazil, this herbicide has previously been reported to provide limited residual weed control, as the degradation half-life (DT₅₀) ranges from 18 – 24 d (Anonymous 2011; Dayan et al. 2009; Dewar 2003; Senseman 2007). Recent research suggests amicarbazone may also have utility for annual bluegrass control in creeping bentgrass putting greens (McCullough et al. 2010; Senseman 2007; Yelverton 2009). McCullough et al. (2010) reported two fall amicarbazone applications at 300 g ai ha⁻¹ on ‘L-93’ creeping bentgrass caused 37 and 8 % injury in Indiana and New Jersey, respectively. When applications were delayed to April, injury was reduced four weeks after treatment at both locations to < 15%. Further, amicarbazone applied in mid-April and early-May at 300 g ha⁻¹ provided 74%

annual bluegrass control at both locations six weeks after the initial treatment. The authors also reported annual bluegrass and ‘L-93’ creeping bentgrass injury increased with temperature from 10 to 30 C across all application rates evaluated (100, 200, 300, 400, and 500 kg ha⁻¹) in controlled environment chambers (McCullough et al. 2010). These findings suggest the usage of amicarbazone on creeping bentgrass putting greens may be limited to the spring season. Yelverton (2009) also found amicarbazone applied in the fall caused unacceptable creeping bentgrass injury, as amicarbazone applied at 260 g ha⁻¹ caused > 95% injury when applied in September, and 0% injury when applications were delayed to April. Further research found four weekly applications of amicarbazone at 49 g ha⁻¹ initiated in late March caused ≤ 11% creeping bentgrass injury and > 80% annual bluegrass control (Yelverton 2010).

As past research has indicated, amicarbazone may be suited for annual bluegrass control in creeping bentgrass putting greens. However, unacceptable creeping bentgrass tolerance has been reported over a range of climatic conditions and geographic expanses. Due to increased turfgrass quality previously observed following paclobutrazol applications, treatment regimens including this plant growth regulator and amicarbazone may reduce creeping bentgrass injury, while providing acceptable annual bluegrass control. The objective of this research was to determine the utility of various amicarbazone and paclobutrazol spring treatment regimens for annual bluegrass control in creeping bentgrass putting greens

Materials and Methods

Experiments were conducted in spring 2010 and 2011 throughout North Carolina to evaluate annual bluegrass control and creeping bentgrass tolerance to treatment regimens including amicarbazone and paclobutrazol. Annual bluegrass control trials were conducted at the following locations: Occoneechee Golf Club (Hillsborough, NC), Prestonwood Country Club (Cary, NC), and the Sandhills Research Station (Jackson Springs, NC). All locations consisted of 'Penncross' creeping bentgrass with natural annual bluegrass infestations. Creeping bentgrass tolerance trials were conducted on a contiguous 929 m² block comprised of four varieties at the Lake Wheeler Turf Field Laboratory (Raleigh, NC). Evaluated creeping bentgrass varieties included: 'A1', 'A4', 'L-93', and 'Crenshaw'. All trials were initiated from March 5th to 10th in both years and experimental units were managed as a golf course putting green with respect to growth medium (currently recommended United States Golf Association rootzone mixture), mowing height (\approx 3.5 mm), irrigation (provided in supplement to rainfall), and fertility (220 to 293 kg N ha⁻¹ yr⁻¹).

Treatment (Trt.) regimens included amicarbazone (Amicarbazone 70 WG[®], Arysta LifeScience Corporation, Cary, NC) applied at 49, 65, or 92 g a.i. ha⁻¹, and paclobutrazol (Trimmit 2 SC[®], Syngenta Crop Protection, Incorporated, Greensboro, NC) applied at 70, 140, or 280 g a.i. ha⁻¹ as stand-alone treatments, tank-mixtures, or in tandem with both compounds applied separately at varying rates and sequential timings (Table 1). All amicarbazone treatments included a non-ionic surfactant (Induce[®], Helena Chemical Company, Collierville, TN) at 0.25% vol vol⁻¹. Foliar broadcast treatments were applied

with a CO₂-pressurized sprayer comprised of three, 8002 XR flat fan nozzles (TeeJet[®] flat-fan nozzles, Spraying Systems Co., Wheaton IL) on 25 cm spacings. Treatments were applied in a water carrier volume of 304 L ha⁻¹ to experimental units measuring 2.0 m². To conform to suggested paclobutrazol application instructions, all trial locations were irrigated with 63,437 L ha⁻¹ within 24 h following applications (Anonymous 2012). Experimental designs were randomized complete blocks with four replicates in both years.

Annual bluegrass cover was visually estimated 1, 2, 3, 4, 6, 8, and 12 weeks after initial treatments (WAIT) on a 0 to 100 % scale, where 0 equaled no cover and 100 equaled complete plot cover. Annual bluegrass control was determined from percent coverage reduction relative to the nontreated using the following equation:

$$[1] \quad \% \text{ control} = [((C_{NT} - C_T) / C_{NT}) \times 100]$$

where C_T and C_{NT} equaled annual bluegrass coverage in a treated and nontreated plot, respectively. Creeping bentgrass quality and injury were rated biweekly following trial initiation. Quality was rated on a 1 to 9 scale, where 1 equaled complete turfgrass death, 7 equaled nontreated turfgrass, and 9 equaled optimal turfgrass growth. Injury was rated on a 0 to 100 scale, where 0 equaled no injury and 100 equaled complete turfgrass death. Based on practical turfgrass management expectations, creeping bentgrass tolerance was considered unacceptable when quality and injury ratings were < 6 or > 10 %, respectively. Digital image analysis (DIA) was conducted monthly to determine percent turf coverage using SigmaScan Pro[®] (SigmaScan Pro[®], version 5, Systat Software, Incorporated, Chicago, IL) as

described by Karcher and Richardson (2005). Images were captured over a 0.12 m² area in the center of each plot utilizing a digital camera (Canon PowerShot SD750[®], Canon Incorporated, Lake Success, NY) mounted to a portable light box (NexGen light box, NexGen Turf Research, Albany, OR) equipped with four TCP T² 9W 6500K SpringLamp[®] light bulbs (TCP 40W Spring Lamps[®], TCP, Incorporated, Aurora, OH). Finally, turf canopy spectral reflectance measurements were taken monthly with a FieldScout TCM 500 NDVI Turf Color Meter (Spectrum Technologies, Plainfield, IL) to calculate normalized difference vegetation index (NDVI). Five reflectance measurements, each over a 45 cm² area were recorded along the center of a plot with the device firmly planted to the turf surface. To reduce variation, reflectance measurements were taken between 1400 and 1600 h eastern standard time on days with ≤ 20% cloud cover (Chang et al. 2005). NDVI was calculated with the following equation:

$$[2] \text{ NDVI} = [(R_{850} - R_{660}) / (R_{850} + R_{660})]$$

where R_{660} and R_{850} denote reflectance at 660 and 850 nm, respectively.

After confirming homogeneity of variance, annual bluegrass control and creeping bentgrass tolerance data were subject to analysis of variance ($P = 0.05$) using mixed model methodology (Statistical Analysis Software[®], version 9.2, SAS Institute Incorporated, Cary, NC). Due to inherent differences between the species, annual bluegrass and creeping bentgrass data were analyzed separately. Fixed effects included treatment regimens and creeping bentgrass variety. Experimental runs and annual bluegrass control locations were

considered environments sampled at random as previously described (Blouin et al. 2011; Carmer et al. 1989). Designating experimental run and location as random effects allows for the comparison of treatment regimen means over a range of environments. Significant main effects and interactions are presented accordingly with precedent given to interactions of increasing magnitude (Steele et al. 1997). Means were separated according to Fisher's protected LSD ($P \leq 0.05$). Finally, Pearson correlation coefficients ($P = 0.05$) were determined to quantify the relationship between turf quality and NDVI, as well as turf injury and coverage determined with DIA.

Results and Discussion

Maximum creeping bentgrass injury from select treatment regimens was observed 8 WAIT. Although treatment regimens were not complete at this time, annual bluegrass control data are presented 8 WAIT due to the influence of reduced inter-species competition on creeping bentgrass growth before the onset of stressful summer climatic conditions. Creeping bentgrass recovered to acceptable tolerance standards from most treatment regimens 12 WAIT. Final annual bluegrass control evaluations were conducted 12 WAIT due to confounding climatic conditions on plant growth at later evaluation dates in early summer. Therefore, data from 8 and 12 WAIT are presented and discussed to highlight regimens which provided increased annual bluegrass control compared to current chemical options, and acceptable creeping bentgrass tolerance.

Annual Bluegrass Control

The main effect of treatment regimen was significant for annual bluegrass control 8 and 12 WAIT. All amicarbazone and paclobutrazol stand-alone regimens (Trts. 1 – 6) provided poor control ($\leq 29\%$) 8 WAIT (Table 2). The effect of sequential timing on amicarbazone regimens can be observed when comparing applications at 49 or 92 g ha⁻¹ (Trts. 1 and 3, respectively). Prior to sequential applications 8 WAIT, annual bluegrass control was four-fold higher, while approximately 20% less active ingredient had been delivered to plots from weekly amicarbazone applications at 49 g ha⁻¹ compared to tri-weekly applications at 92 g ha⁻¹. The importance of amicarbazone treatment initiation timing was revealed when comparing early-March applications at 65 g ha⁻¹ (Trt. 2) and early-April applications at 92 g ha⁻¹ (Trt. 3). Despite approximately 30% less active ingredient delivered to plots prior to applications 8 WAIT, annual bluegrass control was nearly four-fold higher when amicarbazone applications were initiated early-March. Poor performance from paclobutrazol regimens is likely explained by the limited application timing window. Previous research found in addition to spring applications, paclobutrazol should be applied in the fall for increased control (Bell et al. 2004; Johnson and Murphy 1995; McCullough et al. 2005).

In general, increased annual bluegrass control was observed 8 WAIT when amicarbazone and paclobutrazol were both utilized in a treatment regimen (Trts. 7 – 12) (Table 2). Compared to amicarbazone applied alone at 65 g ha⁻¹ (Trt. 2), the addition of

paclobutrazol at 140 or 280 g ha⁻¹ (Trts. 10 and 12, respectively) nearly doubled annual bluegrass control. A tank-mixture of amicarbazone at 65 g ha⁻¹ and paclobutrazol at 280 g ha⁻¹ (Trt. 12) provided the highest control (58%) at this evaluation date. On an equivalent active ingredient basis, this monthly-applied tank-mixture (Trt. 12; 58% control) provided significantly greater control than bi-weekly treatments of alternating compounds at identical application rates (Trt. 11; 41% control).

Annual bluegrass control provided by stand-alone amicarbazone treatment regimens 12 WAIT was largely dependent on sequential application interval (Table 2). Benefits observed 8 WAIT from initiating amicarbazone treatments in early-March were negated by the impact of sequential application interval on annual bluegrass control 12 WAIT. Monthly amicarbazone applications at 65 g ha⁻¹ (Trt. 2) and weekly applications at 49 g ha⁻¹ (Trt. 1) provided 41 and 70 % control, respectively. Although phytotoxic effects were observed from monthly applications at 65 g ha⁻¹, annual bluegrass injury declined 7 to 10 d before sequential applications were made (personal observation). Annual bluegrass coverage reduction from paclobutrazol stand-alone treatments was dependent on the amount of active ingredient applied throughout this research, as control more than doubled when experimental units received 840 g ha⁻¹ season⁻¹ compared to 280 g ha⁻¹ season⁻¹. Further, no differences were observed between regimens delivering 840 g ha⁻¹ season⁻¹ over a differing amount of sequential applications (Trts. 5 and 6).

Treatment regimens utilizing amicarbazone and paclobutrazol provided $\geq 72\%$ annual bluegrass control 12 WAIT (Table 2). The addition of paclobutrazol at 140 or 280 g ha⁻¹ to amicarbazone applied at 65 g ha⁻¹ (Trts. 10 and 11, respectively) improved control compared to the amicarbazone stand-alone regimen (Trt. 2). Further, paclobutrazol application rate and timing also impacted control, as the addition of three paclobutrazol applications at 280 g ha⁻¹ provided superior control to six applications at 140 g ha⁻¹. Control from amicarbazone applied at 49 g ha⁻¹ (Trt. 2) also increased with the addition of paclobutrazol applied at 280 g ha⁻¹ (Trt. 9). Finally, the effects of tank-mixing amicarbazone at 65 g ha⁻¹ and paclobutrazol at 280 g ha⁻¹ on control were additive, as the former and latter treatment regimen (Trts. 2 and 6, respectively) provided 41 and 46 % control, respectively, while the tank-mixture regimen (Trt. 12) provided excellent control (91%).

Creeping Bentgrass Tolerance

The main effect of treatment regimen on creeping bentgrass tolerance was significant for all evaluated parameters 8 and 12 WAIT. With the exception of NDVI data from 8 WAIT, the main effect of creeping bentgrass variety was not significant for all evaluated parameters over the course of this research. Due to previous reports of variable NDVI among differing cultivars of cool-season turfgrass species, this main effect will not be further discussed (Bremer et al. 2011a).

In general, creeping bentgrass tolerance to treatment regimens including amicarbazone applied at 49 or 92 g ha⁻¹ was unacceptable 8 WAIT (Table 3). When

comparing regimens including amicarbazone at 49 g ha^{-1} , stand-alone amicarbazone applications (Trt. 1) caused greatest turf injury, as well as lowest turf quality, NDVI, and turf coverage. These findings disagree with initial observations by Yelverton (2010), who reported this regimen caused $\leq 11\%$ creeping bentgrass injury. However, a review of climatic conditions during the amicarbazone application windows made in 2009 by Yelverton (2010) and those in 2010 and 2011 for the course of this research may explain the inconsistent results. Growth of creeping bentgrass growth is severely limited as ambient temperatures rise above 30 C (Carrow 1996; Fry and Huang 2004). Further, amicarbazone applications on creeping bentgrass are not recommended $> 27 \text{ C}$ (Anonymous 2012). With 27 C as a baseline temperature, average hourly temperature recordings at the Lake Wheeler Road Research Station (Raleigh, NC) were subtracted from this value. All positive values (average hourly temperatures $> 27 \text{ C}$) were summed within a given day and presented chronologically (Figure 1). Note in 2010 temperatures were considerably higher at regimen initiation and conclusion compared to 2009 and 2011. In years where this regimen provided acceptable tolerance, prolonged exposure to temperatures $> 27 \text{ C}$ did not occur before, during, or after (within seven days) the application window for this treatment regimen.

Although turf tolerance was still unacceptable 8 WAIT, the addition of paclobutrazol at 140 or 280 g ha^{-1} (Trts. 8 and 9, respectively) to amicarbazone at 49 g ha^{-1} (Trt. 1) improved all evaluated creeping bentgrass parameters (Table 3). It can be speculated that this safening effect is due to increased antioxidant enzyme activity, namely superoxide dismutase (SOD), ascorbate peroxidase (AP), and glutathione reductase (GR), in

paclobutrazol-treated plants (Gillery and Fletcher 1997; Krans and Fletcher 1994). As mentioned previously, amicarbazone controls susceptible plants by blocking electron flow in PSII (Dayan et al. 2009; Senseman 2007). One consequence from this inhibition is the accumulation of singlet oxygen ($^1\text{O}_2$) and hydrogen peroxide (H_2O_2) (Turgeon et al. 2009). These highly reactive oxygen species cause peroxidation of essential membrane lipids and destroy cell membrane integrity, ultimately causing plant death (Hess 2000). Plants utilize numerous enzymes, including SOD, AP, and GR, to avoid oxidative stress caused by various abiotic factors (Scandalios 1993). Although it is not fully understood how SOD scavenges singlet oxygen, Matheson et al. (1975) concluded it was due to its amino acid content, namely histidine, which readily reacts with singlet oxygen (Nilsson et al. 1972). The author concluded histidine in SOD or free solution were equally accessible for singlet oxygen quenching. Previous research has more clearly determined AP and GR effectively neutralize hydrogen peroxide in chloroplasts and mitochondria, respectively (Foyer and Halliwell 1976; Lawlor 2001; Scandalios 1993). Kraus and Fletcher (1994) found paclobutrazol-treated wheat (*Triticum aestivum* L.) increased SOD, AP, and GR activities 16, 32, and 21 % relative to the control. Further, the authors reported enzymatic activity remained constant in treated plants after a 2.5 h period at 50 C, while this heat stress caused > 20% reduction in SOD, AP, and GR activity in nontreated controls. Gilley and Fletcher (1997) also found paclobutrazol enhanced heat tolerance in wheat under similar conditions, as chlorophyll content and the chlorophyll fluorescence ratio were unaffected in treated plants after heat exposure (3 h at 50 C), while nontreated controls were reduced 28 and 58 %, respectively. The fluorescence

ratio quantifies the efficacy of leaf photosynthesis, where decreased values suggest photoinhibitory damage when plants are under stress.

Stand-alone amicarbazone treatment regimens (Trts. 1 to 3) caused varying degrees of unacceptable creeping bentgrass tolerance 12 WAIT (Table 4). Slight injury (12%) was observed from monthly applications at 65 g ha⁻¹ initiated in early-March (Trt. 2), while turf injury was increased and turf quality was reduced more so by applications initiated in early-April. When comparing regimens initiated early-April, amicarbazone applied every third week at 92 g ha⁻¹ (Trt. 3) likely caused more damage to creeping bentgrass 12 WAIT due to an increased amount of total active ingredient applied over the course of the research, as well as a later application date at regimen conclusion (Table 1). Creeping bentgrass tolerance 12 WAIT from all stand-alone paclobutrazol regimens (Trts. 4 to 6) provided comparable to, or improved quality relative to the control. These findings coincide with previous reports of increased creeping bentgrass quality following spring paclobutrazol applications (Bell et al. 2004; Fagerness et al. 2000; Koski 1997). All treatment regimens including amicarbazone and paclobutrazol (Trts. 7 to 12) provided acceptable creeping bentgrass tolerance 12 WAIT; however, regimens including amicarbazone applied at 49 g ha⁻¹ caused slightly lower turf quality ratings (6.8) compared to 65 g ha⁻¹ (7.6) when averaged over paclobutrazol at 140 or 280 g ha⁻¹.

Correlation of Creeping Bentgrass Ratings

Pearson correlation coefficients were determined on creeping bentgrass tolerance data 8 and 12 WAIT to quantify the relationship between turf quality and NDVI, as well as turf injury and green foliage coverage determined by DIA. Visual estimations of turf quality and NDVI showed a strong positive relationship 8 WAIT ($r = 0.81$; $P < 0.0001$) and 12 WAIT ($r = 0.77$; $P < 0.0001$), indicating turf quality increased with NDVI (Table 5). These findings coincide with previous reports by Bremer et al. (2011b) and Keskin et al. (2008), as correlations (r) between visual turf quality and NDVI determined by the research groups were > 0.82 and $= 0.93$, respectively. Visual turf injury estimations and green foliage coverage determined by DIA showed a strong negative relationship 8 WAIT ($r = -0.86$; $P < 0.0001$) and 12 WAIT ($r = -0.72$; $P < 0.0001$), indicating turf injury increased as green turf coverage determined by DIA decreased. Previous research by Lewis et al. (2010) also found a strong negative correlation between visual estimates of turf injury and DIA, as correlations (r) for zoysiagrass (*Zoysia japonica* Steud.) and bermudagrass (*Cynodon dactylon* L.) data were -0.71 and -0.85 , respectively. The strong correlations determined from this research further supports human-generated ratings compliment non-subjective measurements and when used appropriately, have utility in field-based turfgrass research (Yelverton et al. 2009).

Research Implications

Results from this research indicate amicarbazone applied alone to creeping bentgrass putting greens can be inconsistent, likely dependent on environmental conditions.

Acceptable turfgrass tolerance was observed with three monthly applications at 65 g ha⁻¹ initiated in early-March (Trt.2); however, annual bluegrass control was poor 8 and 12 WAIT (22 and 41 %, respectively). Amicarbazone treatment regimens initiated early-April (Trts. 1 and 3) provided > 70 % annual bluegrass control 12 WAIT, but caused unacceptable creeping bentgrass injury. Although initiation times varied, unacceptable creeping bentgrass tolerance may be partially due to residual amicarbazone activity, as the time interval between sequential applications of amicarbazone applied at 65 g ha⁻¹ (Trt. 2) was greater than the other two stand-alone regimens (Trts. 1 and 3), allowing more time for amicarbazone degradation between applications. Therefore, stand-alone amicarbazone applications should only be made when: 1) creeping bentgrass root systems are well developed; 2) climatic conditions are optimal for creeping bentgrass growth; and 3) creeping bentgrass has not recently (7 – 14 d) been subjected to periods of environmental stress.

The addition of paclobutrazol to amicarbazone regimens at 49 or 65 g ha⁻¹ improved annual bluegrass control and creeping bentgrass tolerance. Therefore, treatment regimens utilizing both compounds are recommended for turf managers. Regimens providing > 70% annual bluegrass control 12 WAIT and acceptable creeping bentgrass tolerance over the course of this research included: monthly amicarbazone applications at 65 g ha⁻¹ with paclobutrazol applied at 140 or 280 g ha⁻¹. Compared to all other regimens, a tank-mixture

of amicarbazone at 65 g ha⁻¹ with paclobutrazol at 280 g ha⁻¹ applied monthly provided the highest annual bluegrass control, as well as acceptable creeping bentgrass tolerance 8 and 12 WAIT. Further, this regimen was completed with the fewest total applications (three applications) compared to all other regimens including amicarbazone and paclobutrazol applied at 140 or 280 g ha⁻¹ (\geq six applications). Aside from a weed control and turfgrass tolerance perspective, reduced applicator/patron exposure and fossil fuel consumption make this regimen superior to all others evaluated in this research. Future research should aim to determine the physiological impacts of paclobutrazol on creeping bentgrass previously treated with PSII-inhibiting herbicides.

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Table 1. Amicarbazone and paclobutrazol treatment regimens for annual bluegrass control in creeping bentgrass putting greens, 2010 and 2011.^a

| Trt. | g ha ⁻¹ | | Application dates | | | | | | | |
|------|--------------------|-----|-------------------|-------|--------|--------|--------|--------|--------|-------|
| | A ^b | P | 03-05 | 03-19 | 04-02 | 04-09 | 04-16 | 04-23 | 04-30 | 05-14 |
| 1 | 49 | --- | --- | --- | A | A | A | A | --- | --- |
| 2 | 65 | --- | A | --- | A | --- | --- | --- | A | --- |
| 3 | 92 | --- | --- | --- | A | --- | --- | A | --- | A |
| 4 | --- | 70 | --- | --- | P | P | P | P | --- | --- |
| 5 | --- | 140 | P | P | P | --- | P | --- | P | P |
| 6 | --- | 280 | P | --- | P | --- | --- | --- | P | --- |
| 7 | 49 | 70 | --- | --- | P tm A | P tm A | P tm A | P tm A | --- | --- |
| 8 | 49 | 140 | P | P | P fb A | A | P fb A | A | P | P |
| 9 | 49 | 280 | P | --- | P fb A | A | A | A | P | --- |
| 10 | 65 | 140 | P fb A | P | P fb A | --- | P | --- | P fb A | P |
| 11 | 65 | 280 | P | A | P | --- | A | --- | P | A |
| 12 | 65 | 280 | P tm A | --- | P tm A | --- | --- | --- | P tm A | --- |
| NT | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |

^a Abbreviations: g ha⁻¹, grams active ingredient per hectare; A, amicarbazone; P, paclobutrazol; Trt, treatment; tm, tank-mixed; fb, followed by; NT, non-treated.

^b All amicarbazone treatments included a non-ionic surfactant at 0.25% vol vol⁻¹.

Table 2. Effect of treatment regimen on annual bluegrass control 8 and 12 WAIT.^a

| Trt. | Description ^c | Control ^b | |
|----------------|-------------------------------|----------------------|---------|
| | | 8 WAIT | 12 WAIT |
| | | % | |
| 1 | A (49 g ♦ 4) ^d | 24 | 70 |
| 2 | A (65 g ♦ 3) | 22 | 41 |
| 3 | A (92 g ♦ 3) | 6 | 90 |
| 4 | P (70 g ♦ 4) | 24 | 20 |
| 5 | P (140 g ♦ 6) | 28 | 42 |
| 6 | P (280 g ♦ 3) | 29 | 46 |
| 7 | P (70 g ♦ 4) tm A (49 g ♦ 4) | 37 | 74 |
| 8 | P (140 g ♦ 6) fb A (49 g ♦ 4) | 32 | 83 |
| 9 | P (280 g ♦ 3) fb A (49 g ♦ 4) | 38 | 86 |
| 10 | P (140 g ♦ 6) fb A (65 g ♦ 3) | 40 | 72 |
| 11 | P (280 g ♦ 3) fb A (65 g ♦ 3) | 41 | 89 |
| 12 | P (280 g ♦ 3) tm A (65 g ♦ 3) | 58 | 91 |
| LSD (P ≤ 0.05) | | 15 | 14 |

^a Abbreviations: WAIT, week after initial treatment; Trt, treatment; A, amicarbazone; P, paclobutrazol; tm, tank-mixed; fb, followed by; LSD, least significant difference.

^b Control determined by cover reduction relative to the nontreated on a 0 – 100 % scale; where 0 equals no cover reduction and 100 equals complete cover reduction. Cover reduction = $\left(\frac{C_{NT} - C_T}{C_{NT}} \right) \times 100$; where C_T and C_{NT} equals annual bluegrass coverage in a treated and nontreated plot, respectively.

^c All amicarbazone treatments included a non-ionic surfactant at 0.25% vol vol⁻¹.

^d Active ingredient (g ha⁻¹ ♦ number of applications).

Table 3. Effect of treatment regimen on creeping bentgrass quality, NDVI, injury, and coverage 8 WAIT.^{a, b}

| Trt. | Description ^c | Quality ^d | NDVI ^e | Injury ^f | Coverage ^g |
|----------------|-------------------------------|----------------------|-------------------|---------------------|-----------------------|
| | | — (1 – 9) — | — (0 – 1) — | % | |
| 1 | A (49 g ♦ 4) ^h | 4.0 | 0.639 | 33 | 58 |
| 2 | A (65 g ♦ 3) | 7.3 | 0.775 | 4 | 95 |
| 3 | A (92 g ♦ 3) | 3.3 | 0.685 | 20 | 71 |
| 4 | P (70 g ♦ 4) | 6.6 | 0.769 | 8 | 98 |
| 5 | P (140 g ♦ 6) | 8.0 | 0.794 | 0 | 98 |
| 6 | P (280 g ♦ 3) | 7.9 | 0.794 | 0 | 98 |
| 7 | P (70 g ♦ 4) tm A (49 g ♦ 4) | 4.3 | 0.704 | 20 | 66 |
| 8 | P (140 g ♦ 6) fb A (49 g ♦ 4) | 6.3 | 0.743 | 14 | 73 |
| 9 | P (280 g ♦ 3) fb A (49 g ♦ 4) | 5.5 | 0.720 | 22 | 76 |
| 10 | P (140 g ♦ 6) fb A (65 g ♦ 3) | 7.8 | 0.770 | 7 | 91 |
| 11 | P (280 g ♦ 3) fb A (65 g ♦ 3) | 7.7 | 0.779 | 8 | 95 |
| 12 | P (280 g ♦ 3) tm A (65 g ♦ 3) | 7.5 | 0.786 | 0 | 96 |
| NT | --- | 7.0 | 0.792 | 0 | 99 |
| LSD (P ≤ 0.05) | | 0.9 | 0.024 | 7 | 6 |

^a Abbreviations: NDVI, normalized difference vegetation index; WAIT, week after initial treatment; Trt, treatment; A, amicarbazone; P, paclobutrazol; tm, tank-mixed; fb, followed by; NT, non-treated; LSD, least significant difference.

^b Data pooled over ‘A1’, ‘A4’, ‘L-93’, and ‘Crenshaw’ creeping bentgrass varieties.

^c All amicarbazone treatments included a non-ionic surfactant at 0.25% vol vol⁻¹.

^d Quality rated on a 1 – 9 scale; where 1 equals complete turfgrass death, 7 equals nontreated turfgrass, and 9 equals optimal turfgrass growth.

^e $NDVI = ((R_{850} - R_{660}) / (R_{850} + R_{660}))$; where R_{850} and R_{660} denote reflectance measurements at 660 and 850 nm, respectively.

^f Injury rated on a 0 – 100 % scale; where 0 equals no injury and 100 equals complete turfgrass death.

^g Coverage measured digitally using SigmaScan[®] software on a 0 – 100 % scale; where 0 equals no green foliage coverage and 100 equals complete green foliage coverage.

^h Active ingredient (g ha⁻¹ ♦ number of applications).

Table 4. Effect of treatment regimen on creeping bentgrass quality, NDVI, injury, and coverage 12 WAIT.^{a, b}

| Trt. | Description ^c | Quality ^d | NDVI ^e | Injury ^f | Coverage ^g |
|-----------------------|-------------------------------|----------------------|-------------------|---------------------|-----------------------|
| | | — (1 – 9) — | — (0 – 1) — | — % — | — % — |
| 1 | A (49 g ♦ 4) ^h | 6.0 | 0.739 | 16 | 92 |
| 2 | A (65 g ♦ 3) | 7.3 | 0.779 | 12 | 100 |
| 3 | A (92 g ♦ 3) | 5.2 | 0.686 | 26 | 72 |
| 4 | P (70 g ♦ 4) | 7.1 | 0.780 | 0 | 99 |
| 5 | P (140 g ♦ 6) | 7.9 | 0.785 | 0 | 100 |
| 6 | P (280 g ♦ 3) | 7.5 | 0.795 | 0 | 99 |
| 7 | P (70 g ♦ 4) tm A (49 g ♦ 4) | 7.0 | 0.777 | 0 | 99 |
| 8 | P (140 g ♦ 6) fb A (49 g ♦ 4) | 7.3 | 0.780 | 0 | 99 |
| 9 | P (280 g ♦ 3) fb A (49 g ♦ 4) | 6.3 | 0.751 | 1 | 98 |
| 10 | P (140 g ♦ 6) fb A (65 g ♦ 3) | 7.6 | 0.781 | 0 | 99 |
| 11 | P (280 g ♦ 3) fb A (65 g ♦ 3) | 7.5 | 0.785 | 0 | 100 |
| 12 | P (280 g ♦ 3) tm A (65 g ♦ 3) | 7.1 | 0.783 | 1 | 99 |
| NT | --- | 7.0 | 0.787 | 0 | 100 |
| LSD ($P \leq 0.05$) | | 0.5 | 0.025 | 6 | 5 |

^a Abbreviations: NDVI, normalized difference vegetation index; WAIT, week after initial treatment; Trt, treatment; A, amicarbazone; P, paclobutrazol; tm, tank-mixed; fb, followed by; NT, non-treated; LSD, least significant difference.

^b Data pooled over ‘A1’, ‘A4’, ‘L-93’, and ‘Crenshaw’ creeping bentgrass varieties.

^c All amicarbazone treatments included a non-ionic surfactant at 0.25% vol vol⁻¹.

^d Quality rated on a 1 – 9 scale; where 1 equals complete turfgrass death, 7 equals nontreated turfgrass, and 9 equals optimal turfgrass growth.

^e $NDVI = ((R_{850} - R_{660}) / (R_{850} + R_{660}))$; where R_{850} and R_{660} denote reflectance measurements at 660 and 850 nm, respectively.

^f Injury rated on a 0 – 100 % scale; where 0 equals no injury and 100 equals complete turfgrass death.

^g Coverage measured digitally using SigmaScan[®] software on a 0 – 100 % scale; where 0 equals no green foliage coverage and 100 equals complete green foliage coverage.

^h Active ingredient (g ha⁻¹ ♦ number of applications).

Table 5. Pearson correlation coefficients for the relationships between visual estimates of creeping bentgrass quality and injury with NDVI and DIA 8 and 12 WAIT.^{a, b}

| | 8 WAIT | | 12 WAIT | |
|----------------------|-------------------|------------------|----------|----------|
| | NDVI ^c | DIA ^d | NDVI | DIA |
| Quality ^e | 0.81 | --- | 0.77 | --- |
| Injury ^f | --- | -0.86 | --- | -0.72 |
| P = | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

^a Abbreviations: NDVI, normalized difference vegetation index; DIA, digital image analysis; WAIT, week after initial treatment.

^b Data pooled over ‘A1’, ‘A4’, ‘L-93’, and ‘Crenshaw’ creeping bentgrass varieties.

^c $NDVI = ((R_{850} - R_{660}) / (R_{850} + R_{660}))$; where R_{850} and R_{660} denote reflectance measurements at 660 and 850 nm, respectively.

^d Coverage measured digitally using SigmaScan[®] software on a 0 – 100 % scale; where 0 equals no green foliage coverage and 100 equals complete green foliage coverage.

^e Quality rated on a 1 – 9 scale; where 1 equals complete turfgrass death, 7 equals nontreated turfgrass, and 9 equals optimal turfgrass growth.

^f Injury rated on a 0 – 100 % scale; where 0 equals no injury and 100 equals complete turfgrass death.

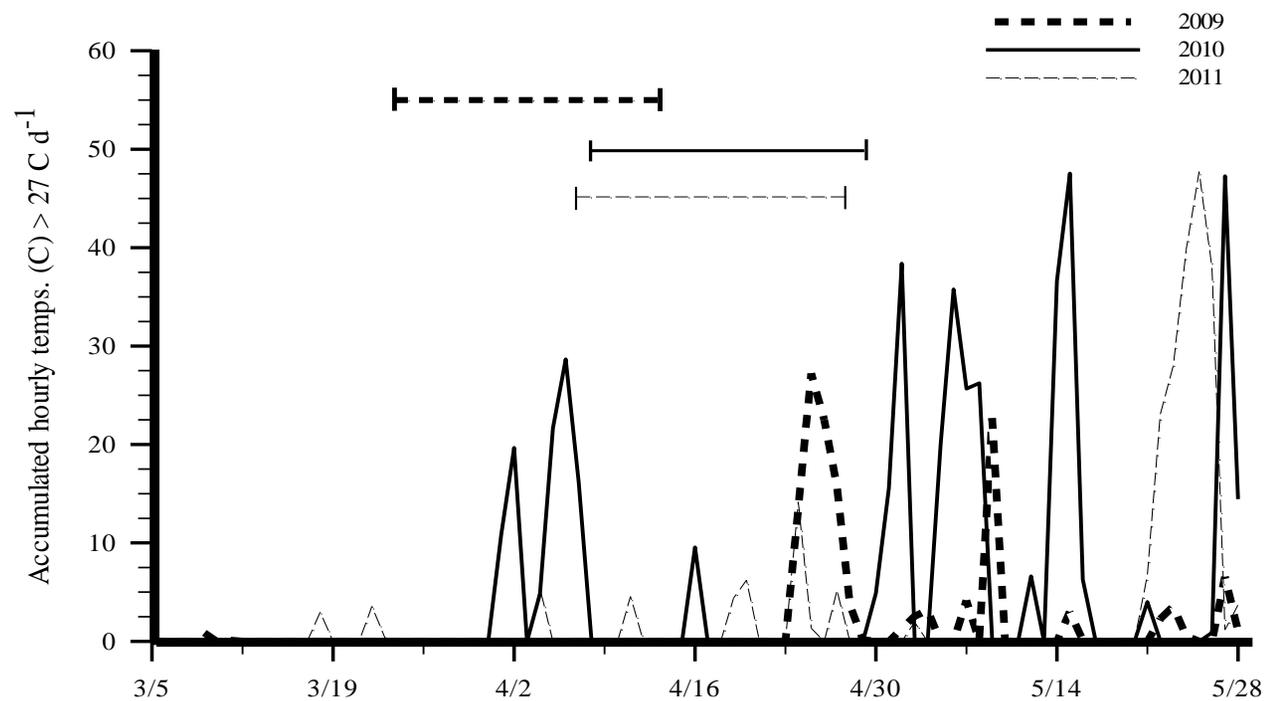


Figure 1. Accumulated temperature over 24 hourly average temperatures above 27 C per day at the Lake Wheeler Turf Field Laboratory, Raleigh, NC. Data presented represents full trial application and evaluation windows of research conducted in 2010 and 2011, compared with 2009 by Yelverton (2010). Capped horizontal lines represent the application window of treatment regimens including four, weekly amicarbazone at 49 g ha⁻¹ in each respective year of research.