

Annual Bluegrass (*Poa annua*) Control in Creeping Bentgrass Putting Greens with Amicarbazone and Paclobutrazol

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Amicarbazone is a photosystem II-inhibiting herbicide recently registered for annual bluegrass control in established turf systems that include creeping bentgrass. However, research to date reveals potential issues with creeping bentgrass tolerance to amicarbazone. Currently, the plant-growth regulator paclobutrazol is widely adopted by turf managers for chemical annual bluegrass suppression in creeping bentgrass putting greens. Field experiments were conducted throughout North Carolina in the spring of 2010 and 2011 to assess treatment regimens that included amicarbazone (49, 65, or 92 g ai ha⁻¹) and paclobutrazol (70, 140, or 280 g ai ha⁻¹) applied alone, as tank-mixtures, or used in tandem, at varying rates and sequential timings for annual bluegrass control in creeping bentgrass putting greens. In general, regimens including both compounds provided greater annual bluegrass control and acceptable turfgrass tolerance compared with stand-alone applications of amicarbazone at 8 and 12 wk after initial treatment (WAIT). When comparing regimens that included amicarbazone at 49 or 65 g ha⁻¹, creeping bentgrass tolerance was greater for the higher application rate applied less frequently. These results indicate amicarbazone usage on creeping bentgrass greens may be beneficially affected with the incorporation of paclobutrazol to treatment regimens because annual bluegrass control with the combination was equal to or greater than stand-alone amicarbazone applications, and creeping bentgrass tolerance was superior 8 and 12 WAIT.

Nomenclature: Amicarbazone; paclobutrazol; annual bluegrass, *Poa annua* L.; creeping bentgrass, *Agrostis stolonifera* L.
Key words: Digital image analysis, injury, quality, turf spectral reflectance, weed control.

Amicarbazone es un herbicida inhibidor del fotosistema II que fue recientemente registrado para el control de *Poa annua* en sistemas de céspedes establecidos incluyendo *Agrostis stolonifera*. Sin embargo, la investigación hasta la fecha revela problemas potenciales con la tolerancia de *A. stolonifera* a amicarbazone. Actualmente, el regulador de crecimiento paclobutrazol es ampliamente adoptado por especialistas en céspedes para la supresión química de *P. annua* en putting greens de *A. stolonifera*. Experimentos de campo fueron realizados en North Carolina en la primavera de 2010 y 2011 para evaluar regímenes con tratamientos que incluyeron amicarbazone (49, 65, ó 92 g ai ha⁻¹) y paclobutrazol (70, 140, ó 280 g ai ha⁻¹), aplicados solos o en mezcla en tanque, o usados en tándem, a dosis variables y en aplicaciones secuenciales para el control de *P. annua* en putting greens de *A. stolonifera*. En general, los regímenes que incluyeron ambos compuestos brindaron un mayor control de *P. annua* y una tolerancia aceptable del césped en comparación con aplicaciones solas de amicarbazone a 8 y 12 semanas después del tratamiento inicial (WAIT). Cuando se compararon regímenes que incluyeron amicarbazone a 49 ó 65 g ha⁻¹, la tolerancia del césped fue mayor cuando las dosis más altas fueron aplicadas en menor frecuencia. Estos resultados indican que el uso de amicarbazone en greens de *A. stolonifera* podría ser afectado beneficiosamente con la incorporación de paclobutrazol porque el control de *P. annua* con la combinación fue igual o mayor que las aplicaciones solas de amicarbazone, y la tolerancia del césped fue superior a 8 y 12 WAIT.

Annual bluegrass is the most troublesome weed in creeping bentgrass throughout the United States (Turgeon et al. 2009). Native to Europe, annual bluegrass is widely considered a troublesome weed species because of its copious seedhead production, poor heat tolerance and cold hardiness, and high wilting tendency (Beard 1970). Annual bluegrass can produce 80 viable seeds inflorescence⁻¹ when grown under low density and may produce seedheads at golf course putting green mowing heights (\approx 3 mm) (Christians 2011; Law et al. 1977; Turgeon et al. 2009). Inflorescence development has a detrimental effect on turfgrass playability, often interfering with ball roll on putting greens (Christians 2011; Vargas and Turgeon 2004). Annual bluegrass sensitivity to environmental conditions also reduces turfgrass aesthetics and functionality because areas infested with this weed are often left discolored

or barren following exposure to warm, dry periods (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 United States (Turgeon 2012). Native to Eurasia, creeping bentgrass forms an extremely fine-textured, dense playing surface (Beard 1973). Although this turf species provides a high-quality playing surface, creeping bentgrass has inherent difficulty surviving in late summer months due to intensive mowing practices coupled with unfavorable environmental conditions common in the southern boundaries of its distribution (Carrow 1996). This is especially problematic for preventing annual bluegrass infestations because the timing of this scenario is followed directly by peak annual bluegrass germination conditions in the fall (Kaminski and Dernoeden 2007; Shem-Tov and Fennimore 2003).

Currently, there are no POST herbicides registered for annual bluegrass control in creeping bentgrass putting greens (Turgeon et al. 2009). Golf course superintendents use plant growth regulators (PGRs), namely flurprimidol and paclobu-

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trazol, to suppress annual bluegrass populations (Turgeon et al. 2009; Vargas and Turgeon 2004). Bell et al. (2004) reported that four paclobutrazol applications applied every 2 wk at 110 g ai ha⁻¹ initiated mid September, followed by five applications beginning in March, reduced annual bluegrass coverage in early April by 79 and 85% relative to the nontreated in 2002 and 2003, respectively. Koski (1997) concluded annual bluegrass control from paclobutrazol applications on creeping bentgrass putting greens was due to differential growth suppression because growth of the former species was suppressed more than the latter was. 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 that during 58 consecutive weekly evaluations, creeping bentgrass quality and density were higher than the control in paclobutrazol-treated plots on 38 (66%) and 35 (60%) rating dates, respectively. To date, evaluations of paclobutrazol for control of *Poa annua* spp. *reptans*, the perennial biotype of annual bluegrass commonly found on putting greens, reveals the need for other chemical options. Johnson and Murphy (1996) reported that three paclobutrazol applications in the spring and fall totaling 2.6 kg ha⁻¹ suppressed the perennial biotype 80% relative to the nontreated control 3 wk after the final treatment; however, suppression declined 23% during the following 13 wk. The authors concluded multiple applications over an indefinite amount of years were required to suppress populations of the perennial biotype to acceptable levels.

Amicarbazone is a photosystem II (PSII)-inhibiting herbicide belonging to the triazolinone herbicide family (Senseman 2007). In 2012, amicarbazone was registered in the United States for annual bluegrass control in creeping bentgrass, including golf course roughs, fairways, and tees (Anonymous 2012). Recent research suggests amicarbazone may also have utility for annual bluegrass control in creeping bentgrass putting greens (Yelverton 2010). Four weekly applications of amicarbazone at 49 g ai ha⁻¹, initiated in late March, provided > 80% annual bluegrass control and ≤ 11% creeping bentgrass injury (Yelverton 2010). However, unacceptable creeping bentgrass tolerance to amicarbazone has been reported over a range of climatic conditions and geographic expanses, with season of application and POST-application climatic conditions having the most notable effects on turf injury (McCullough et al. 2010; Yelverton 2009). Because of increased turfgrass quality previously observed following paclobutrazol applications, treatment regimens combining this PGR and amicarbazone may reduce creeping bentgrass injury while providing acceptable annual bluegrass control. The objective of this research was to determine the efficacy 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 in North Carolina to evaluate annual bluegrass control and

creeping bentgrass tolerance to treatment regimens that included amicarbazone and paclobutrazol. Annual bluegrass control trials were conducted at the following locations: Occaneechee 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 that comprised four varieties at the Lake Wheeler Turf Field Laboratory (Raleigh, NC). Evaluated creeping bentgrass varieties included 'Penn A1', 'Penn A4', 'L-93', and 'Crenshaw'. All trials were initiated from March 5 to March 10 in both years. Trials in 2011 were conducted in untreated areas adjacent to trials from 2010. Experimental units were managed as a golf course putting green with respect to fungicide/insecticide applications, growth medium (currently recommended United States Golf Association root-zone mixture), mowing height (≈ 3.5 mm), irrigation (provided to supplement rainfall), and fertility (220 to 293 kg N ha⁻¹ yr⁻¹). Annual bluegrass seedhead suppressants were not applied to experimental units throughout the research.

Treatment regimens included amicarbazone (Xonerate 70 WG, Arysta LifeScience Corporation, Cary, NC) applied at 49, 65, or 92 g ha⁻¹ and paclobutrazol (Trimmit 2 SC, Syngenta Crop Protection, Inc., Greensboro, NC) applied at 70, 140, or 280 g 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 nonionic surfactant (Induce, Helena Chemical Company, Collierville, TN) at 0.25% v/v. Amicarbazone application rates and timings were modified from previous research evaluating higher rates with fewer applications per season, which caused unacceptable creeping bentgrass injury (Yelverton 2009, 2010). 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 trials were irrigated with 53,000 L ha⁻¹ within 24 h after applications (Anonymous 2011). A randomized complete-block experimental design with four replicates, including a nontreated control, was used for this research.

Annual bluegrass cover was visually estimated at 1, 2, 3, 4, 6, 8, and 12 wk 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 by calculating the change in annual bluegrass cover relative to the nontreated control within a given replicate using the following equation:

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

where C_T and C_{NT} equaled annual bluegrass coverage in a treated and nontreated plot, respectively. Creeping bentgrass quality and injury were rated every 2 wk following trial initiation. Quality was rated on a 1 to 9 scale, where 1 equaled

Table 1. Amicarbazone and paclobutrazol treatment regimens for annual bluegrass control and creeping bentgrass tolerance trials, 2010 and 2011.^a

A ^b	P	Application dates ^c							
		March 5	March 19	April 2	April 9	April 16	April 23	April 30	May 14
—	g ha ⁻¹	—	—	—	—	—	—	—	—
49	—	—	—	A	A	A	A	—	—
65	—	A	—	A	—	—	—	A	—
92	—	—	—	A	—	—	A	—	A
—	70	—	—	P	P	P	P	—	—
—	140	P	P	P	—	P	—	P	P
—	280	P	—	P	—	—	—	P	—
49	70	—	—	P tm A	P tm A	P tm A	P tm A	—	—
49	140	P	P	P fb A	A	P fb A	A	P	P
49	280	P	—	P fb A	A	A	A	P	—
65	140	P fb A	P	P fb A	—	P	—	P fb A	P
65	280	P	A	P	—	A	—	P	A
65	280	P tm A	—	P tm A	—	—	—	P tm A	—
Nontreated	—	—	—	—	—	—	—	—	—

^a Abbreviations: A, amicarbazone; P, paclobutrazol; tm, tank-mixed; fb, followed by.

^b All amicarbazone treatments included a nonionic surfactant at 0.25% v/v.

^c All locations in both years were treated within a 5-d period following the presented date.

low-quality turf and 9 equaled high-quality turf. Injury was rated on a 0 to 100% scale relative to the nontreated control, 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 the percentage of creeping bentgrass coverage using SigmaScan Pro (Version 5, Systat Software, Inc., Chicago, IL) as described by Karcher and Richardson (2005). Images were captured over a 1,200-cm² area in the center of each plot using a digital camera (Canon PowerShot SD750, Canon USA Inc., Lake Success, NY) mounted to a portable light box (NexGen light box, NexGen Turf Research, Albany, OR) equipped with four T² 9W 6500K SpringLamp light bulbs (TCP 40W Spring Lamps, TCP, Inc., Aurora, OH). Finally, turf canopy spectral-reflectance measurements were taken monthly with a FieldScout TCM 500 NDVI Turf Color Meter (Spectrum Technologies, Inc. Plainfield, IL) to calculate normalized difference vegetation index (NDVI). Five 45-cm² reflectance measurements 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 2:00 P.M. and 4:00 P.M. eastern standard time on days with ≤ 20% cloud cover (Chang et al. 2005). NDVI was calculated with the following equation:

$$NDVI = (R_{850} - R_{660}) / (R_{850} + R_{660}) \quad (2)$$

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 ANOVA ($P = 0.05$) using mixed-model methodology (SAS Software, version 9.2, SAS Institute Inc., Cary, NC). Because of 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 that time, annual bluegrass control data are presented 8 WAIT because of the influence of reduced interspecies competition on creeping bentgrass growth before the onset of stressful summer climatic conditions. Creeping bentgrass tolerance recovered to acceptable levels from most treatment regimens 12 WAIT. Final annual bluegrass control evaluations were conducted 12 WAIT because of 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 that provided (1) increased annual bluegrass control compared with current chemical options, and (2) acceptable creeping bentgrass tolerance.

Annual Bluegrass Control. The main effect of treatment regimen was significant for annual bluegrass control 8 and 12 WAIT. Although not all applications had been made for regimens that included amicarbazone at 92 g ha⁻¹ or paclobutrazol at 140 g ha⁻¹, all stand-alone amicarbazone and paclobutrazol regimens provided poor control (≤ 29%) 8 WAIT (Table 2). The effect of sequential timing on

Table 2. Effect of treatment regimen on annual bluegrass control at 8 and 12 wk after initial treatment.^{a,b}

Treatment regimen ^d	Control ^c	
	8 WAIT	12 WAIT
g ai ha ⁻¹ × No. of treatments	%	
A (49 × 4)	24	70
A (65 × 3)	22	41
A (92 × 3)	6	90
P (70 × 4)	24	20
P (140 × 6)	28	42
P (280 × 3)	29	46
P (70 × 4) tm A (49 × 4)	37	74
P (140 × 6) fb A (49 × 4)	32	83
P (280 × 3) fb A (49 × 4)	38	86
P (140 × 6) fb A (65 × 3)	40	72
P (280 × 3) fb A (65 × 3)	41	89
P (280 × 3) tm A (65 × 3)	58	91
LSD _{0.05}	15	14

^a Abbreviations: WAIT, weeks after initial treatment; A, amicarbazone; P, paclobutrazol; tm, tank-mixed; fb, followed by.

^b Data were pooled over year and location.

^c Control was determined by cover reduction relative to the nontreated on a 0 to 100% scale, where 0% was no cover reduction and 100% was complete cover reduction.

^d All amicarbazone treatments included a nonionic surfactant at 0.25% v/v.

amicarbazone regimens can be observed when comparing applications at 49 or 92 g ha⁻¹. Before sequential applications 8 WAIT, annual bluegrass control was fourfold higher, whereas approximately 20% less ai had been delivered to plots from weekly amicarbazone applications at 49 g ha⁻¹ compared with applications every 3 wk at 92 g ha⁻¹. The importance of amicarbazone treatment initiation timing was observed when comparing early March applications at 65 g ha⁻¹ and early April applications at 92 g ha⁻¹. Despite approximately 30% less ai delivered to plots before applications 8 WAIT, annual bluegrass control was nearly fourfold higher when amicarbazone applications were initiated in early March compared with those initiated in April. Poor performance from paclobutrazol regimens is likely explained by the limited application window. Previous research found that, in addition to spring applications, paclobutrazol should be applied in the fall for increased control (Bell et al. 2004).

Annual bluegrass control 8 WAIT from regimens that included amicarbazone at 49 g ha⁻¹ was ≤ 38%, with no differences detected between the stand-alone amicarbazone regimen and others incorporating paclobutrazol tank-mixed or applied sequentially (Table 2). Annual bluegrass control from amicarbazone at 65 g ha⁻¹ increased with the addition of paclobutrazol to the regimen. Compared with amicarbazone applied alone at 65 g ha⁻¹, the addition of paclobutrazol at 140 or 280 g ha⁻¹ nearly doubled annual bluegrass control. A tank-mixture of amicarbazone at 65 g ha⁻¹ and paclobutrazol at 280 g ha⁻¹ provided the highest control (58%) 8 WAIT. On an equivalent ai basis, this monthly applied tank-mixture (58% control) provided significantly greater control than bi-weekly treatments of alternating compounds at identical application rates (41% control).

Annual bluegrass control from 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 effect of sequential-application interval on annual bluegrass control 12 WAIT. Monthly amicarbazone applications at 65 g ha⁻¹ and weekly applications at 49 g ha⁻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 ai applied throughout this research, as control more than doubled when experimental units received 840 g ha⁻¹ season⁻¹ compared with 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.

Treatment regimens that included amicarbazone and paclobutrazol provided ≥ 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 improved control compared with the amicarbazone stand-alone regimen. Further, paclobutrazol application rate and timing also affected control because 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 also increased with the addition of paclobutrazol applied at 280 g ha⁻¹. Finally, the effects of tank-mixing or sequentially applying amicarbazone at 65 g ha⁻¹ and paclobutrazol at 280 g ha⁻¹ on control were additive because the former and latter treatment regimen provided 41 and 46% control, respectively, whereas the tank-mixture and the sequentially applied regimens provided 91 and 89% control, respectively.

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. Because of 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 of treatment regimens that included amicarbazone applied at 49 or 92 g ha⁻¹ was unacceptable 8 WAIT (Table 3). However, the addition of paclobutrazol at 140 or 280 g ha⁻¹ to amicarbazone at 49 g ha⁻¹ increased turf quality and reduced turf injury. These findings differ from the initial observations by Yelverton (2010), who reported four weekly amicarbazone applications beginning in late March at 49 g ha⁻¹ caused ≤ 11% creeping bentgrass injury. A review of climatic conditions during the amicarbazone application windows of 2009 by Yelverton (2010) and those in 2010 and 2011 for the course of this research may explain the contrasting results. Because of nonsustainable photorespiration rates, creeping bentgrass growth is severely limited as ambient temperatures

Table 3. Effect of treatment regimen on creeping bentgrass quality, normalized difference vegetation index, injury, and coverage 8 wk after initial treatment.^{a-c}

Treatment regimen ^d	Quality ^e	NDVI ^f	Injury ^g	Coverage ^h
g ai ha ⁻¹ × No. of treatments	1–9	0–1	%	
A (49 × 4)	4.0	0.639	33	58
A (65 × 3)	7.3	0.775	4	95
A (92 × 3)	3.3	0.685	20	71
P (70 × 4)	6.6	0.769	8	98
P (140 × 6)	8.0	0.794	0	98
P (280 × 3)	7.9	0.794	0	98
P (70 × 4) tm A (49 × 4)	4.3	0.704	20	66
P (140 × 6) fb A (49 × 4)	6.3	0.743	14	73
P (280 × 3) fb A (49 × 4)	5.5	0.720	22	76
P (140 × 6) fb A (65 × 3)	7.8	0.770	7	91
P (280 × 3) fb A (65 × 3)	7.7	0.779	8	95
P (280 × 3) tm A (65 × 3)	7.5	0.786	0	96
Nontreated	7.0	0.792	0	99
LSD _{0.05}	0.9	0.024	7	6

^a Abbreviations: NDVI, normalized difference vegetation index; WAIT, weeks after initial treatment; A, amicarbazone; P, paclobutrazol; tm, tank-mixed; fb, followed by.

^b Research was conducted at the Lake Wheeler Turf Field Laboratory, Raleigh, NC.

^c Data were pooled over year and variety.

^d All amicarbazone treatments included a nonionic surfactant at 0.25% v/v.

^e Quality was rated on a scale of 1 to 9, where 1 was complete turfgrass death and 9 was ideal turfgrass growth.

^f $NDVI = [(R_{850} - R_{660}) / (R_{850} + R_{660})]$, where R_{660} and R_{850} are reflectance at 660 and 850 nm, respectively.

^g Injury was rated on a 0 to 100% scale, where 0% was no injury and 100% was complete turfgrass death.

^h Coverage was determined with SigmaScan on a 0 to 100% scale, where 0 was no green turf coverage and 100% was complete green turf coverage.

rise above 30 C (Carrow 1996). Further, amicarbazone applications on creeping bentgrass are not recommended at > 27 C (Anonymous 2012). To present the accumulated temperature above that baseline value within a day, 27 C was subtracted from average hourly temperature recordings at the Lake Wheeler Road Research Station (Raleigh, NC). All positive values (average hourly temperatures > 27 C) were summed within a given day and presented chronologically (Figure 1). Note that, in 2010, temperatures were considerably higher at regimen initiation and conclusion compared with the temperatures in 2009 and 2011. In years where this regimen provided acceptable tolerance, prolonged exposure to temperatures > 27 C did not occur before, during, or after (within 7 d) the application window for that treatment regimen.

Although turf tolerance was still unacceptable 8 WAIT, the addition of paclobutrazol at 140 or 280 g ha⁻¹ to amicarbazone at 49 g ha⁻¹ improved all evaluated creeping bentgrass parameters (Table 3). One hypothesis for this safening effect is increased antioxidant activity, namely superoxide dismutase (SOD), ascorbate peroxidase (AP), and glutathione reductase (GR), in paclobutrazol-treated plants. As mentioned previously, amicarbazone controls susceptible plants by blocking electron flow in PSII (Dayan et al. 2009; Senseman 2007). One consequence of this inhibition is the accumulation of singlet oxygen (¹O₂) and hydrogen peroxide (H₂O₂) (Turgeon et al. 2009). These highly reactive oxygen species cause peroxidation of essential

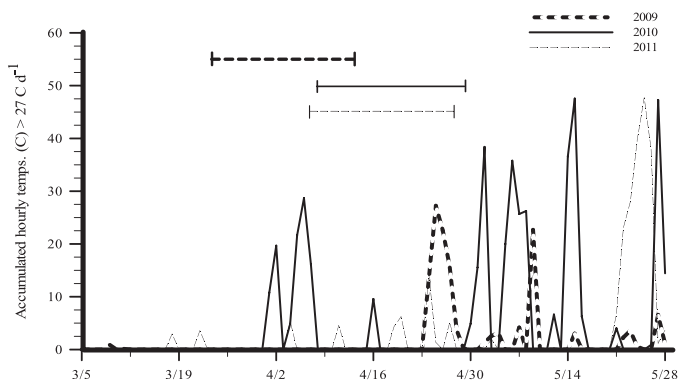


Figure 1. Accumulated temperature over 24 hourly average temperatures above 27 C d⁻¹ at the Lake Wheeler Turf Field Laboratory, Raleigh, NC. Data presented represents full trial application and evaluation windows of research conducted in 2010 and 2011 compared with 2009 research presented by Yelverton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

membrane lipids and destroy cell membrane integrity, ultimately causing plant death (Hess 2000). Plants use 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 ¹O₂, Matheson et al. (1975) concluded it was due to its amino acid content, namely histidine, which readily reacts with ¹O₂ (Nilsson et al. 1972). Previous research has more clearly determined that AP and GR effectively neutralize H₂O₂ in chloroplasts and mitochondria, respectively (Foyer and Halliwell 1976; Scandalios 1993). Kraus and Fletcher (1994) found paclobutrazol-treated wheat (*Triticum aestivum* L.) increased SOD, AP, and GR activities 16, 32, and 21%, respectively, relative to the control. Further, the authors reported enzymatic activity remained constant in treated plants after a 2.5-h period at 50 C, whereas that 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 because chlorophyll content and the chlorophyll fluorescence ratio were unaffected in treated plants after heat exposure (3 h at 50 C), whereas nontreated controls were reduced 28 and 58%, respectively. The chlorophyll fluorescence ratio quantifies the efficacy of leaf photosynthesis, where decreased values suggest photoinhibitory damage when plants are under stress.

Stand-alone amicarbazone treatment regimens caused varying degrees of unacceptable creeping bentgrass response 12 WAIT (Table 4). Slight injury (12%) was observed from monthly applications at 65 g ha⁻¹ initiated in early March, whereas turf injury was increased and turf quality was reduced by applications initiated in early April. When comparing regimens initiated in early April (Table 1), amicarbazone applied every third week at 92 g ha⁻¹ likely caused more damage to creeping bentgrass 12 WAIT because of an increased amount of total ai applied over the course of the research and because of a later application date at the regimen conclusion when climatic conditions were less favorable for optimal turfgrass growth (Figure 1). Creeping bentgrass

Table 4. Effect of treatment regimen on creeping bentgrass quality, normalized difference vegetation index, injury, and coverage 12 wk after initial treatment.^{a-c}

Treatment regimen ^d	Quality ^e	NDVI ^f	Injury ^g	Coverage ^h
g ai ha ⁻¹ × No. of treatments	1 to 9	0 to 1	— % —	
A (49 × 4)	6.0	0.739	16	92
A (65 × 3)	7.3	0.779	12	100
A (92 × 3)	5.2	0.686	26	72
P (70 × 4)	7.1	0.780	0	99
P (140 × 6)	7.9	0.785	0	100
P (280 × 3)	7.5	0.795	0	99
P (70 × 4) tm A (49 × 4)	7.0	0.777	0	99
P (140 × 6) fb A (49 × 4)	7.3	0.780	0	99
P (280 × 3) fb A (49 × 4)	6.3	0.751	1	98
P (140 × 6) fb Ai (65 × 3)	7.6	0.781	0	99
P (280 × 3) fb A (65 × 3)	7.5	0.785	0	100
P (280 × 3) tm A (65 × 3)	7.1	0.783	1	99
Nontreated	7.0	0.787	0	100
LSD _{0.05}	0.5	0.025	6	5

^a Abbreviations: NDVI, normalized difference vegetation index; WAIT, weeks after initial treatment; A, amicarbazone; P, paclobutrazol; tm, tank-mixed; fb, followed by.

^b Research was conducted at the Lake Wheeler Turf Field Laboratory, Raleigh, NC.

^c Data were pooled over year and variety.

^d All amicarbazone treatments included a nonionic surfactant at 0.25% v/v.

^e Quality was rated on a scale of 1 to 9, where 1 was complete turfgrass death and 9 was ideal turfgrass growth.

^f $NDVI = [(R_{850} - R_{660}) / (R_{850} + R_{660})]$, where R_{660} and R_{850} are reflectance at 660 and 850 nm, respectively.

^g Injury was rated on a scale of 0 to 100%, where 0% was no injury and 100% was complete turfgrass death.

^h Coverage was determined with SigmaScan on a scale of 0 to 100%, where 0% was no green turf coverage and 100% was complete green turf coverage.

tolerance 12 WAIT from all stand-alone paclobutrazol regimens was comparable to, or had improved quality, relative to the nontreated control. These findings are similar to previous reports of increased creeping bentgrass quality following spring paclobutrazol applications (Bell et al. 2004; Koski 1997).

All treatment regimens that included amicarbazone and paclobutrazol provided acceptable creeping bentgrass tolerance 12 WAIT; however, regimens that included amicarbazone applied at 49 g ha⁻¹ caused slightly lower turf-quality ratings (6.8) compared with 65 g ha⁻¹ (7.6) when averaged over paclobutrazol applications at 140 or 280 g ha⁻¹ (Table 4). Further, tank-mixing paclobutrazol with amicarbazone at 49 and 65 g ha⁻¹ reduced turf injury ≥ 12%. Creeping bentgrass responded similarly to regimens that included amicarbazone and paclobutrazol applied as tank-mixtures or applied sequentially on this evaluation date (12 WAIT).

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 agree with previous reports by Bremer et al. (2011b) and Keskin et al. (2008) because

Table 5. Pearson correlation coefficients for the relationships between visual estimates of creeping bentgrass quality and injury with normalized difference vegetation index and digital image analysis at 8 and 12 wk after initial treatment.^{a-c}

	8 WAIT		12 WAIT	
	NDVI ^d	DIA ^e	NDVI	DIA
Quality ^f	0.81	—	0.77	—
Injury ^g	—	-0.86	—	-0.72
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

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

^b Research was conducted at the Lake Wheeler Turf Field Laboratory, Raleigh, NC.

^c Data were pooled over year and variety.

^d $NDVI = [(R_{850} - R_{660}) / (R_{850} + R_{660})]$, where R_{660} and R_{850} are reflectance at 660 and 850 nm, respectively.

^e DIA coverage was determined with SigmaScan on a scale of 0 to 100% scale, where 0% was no green turf coverage and 100% was complete green turf coverage.

^f Quality was rated on a scale of 1 to 9, where 1 was complete turfgrass death and 9 was ideal turfgrass growth.

^g Injury was rated on a 0 to 100% scale, where 0% was no injury and 100% was complete turfgrass death.

correlations between visual turf quality and NDVI determined by those research groups were $r > 0.82$ and $r = 0.93$, respectively. Visual turf-injury estimations and green foliage coverage determined by DIA in our research 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, as 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 because correlations for zoysiagrass (*Zoysia japonica* Steud.) and bermudagrass [*Cynodon dactylon* (L.) Pers.] data were $r = -0.71$ and $r = -0.85$, respectively. The strong correlations demonstrate that visual ratings and nonsubjective measurements complement each other and, when used appropriately, both have utility in turfgrass research (Hoyle et al. 2013).

Research Implications. Results from this research indicate amicarbazone applied alone to creeping bentgrass putting greens can cause unacceptable injury. Acceptable turfgrass tolerance was observed with three monthly applications at 65 g ha⁻¹ initiated in early March; however, annual bluegrass control was poor 8 and 12 WAIT (22 and 41%, respectively). Amicarbazone treatment regimens initiated in early April 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 because the interval between sequential applications of amicarbazone applied at 65 g ha⁻¹ was greater than was the interval of the other two stand-alone regimens, allowing more time for amicarbazone degradation between applications. Therefore, stand-alone amicarbazone applications should only be made when (1) creeping bentgrass systems are well established, (2) climatic conditions are optimal for creeping

bentgrass growth, and (3) creeping bentgrass has not recently (7 to 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 that use 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 with 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 and acceptable creeping bentgrass tolerance 8 and 12 WAIT. Further, this regimen was completed with the fewest total applications (three applications) compared with all other regimens that included amicarbazone and paclobutrazol applied at 140 or 280 g ha⁻¹ (six applications or more). 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 effects of paclobutrazol on creeping bentgrass previously treated with PSII-inhibiting herbicides.

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