

This is a “preproof” accepted article for Weed Science. This version may be subject to change in the production process, *and does not include access to supplementary material*.

DOI: 10.1017/wet.2024.100

## **Evaluation of spring-applied endothall for annual bluegrass control in warm-season turf**

John M. Peppers<sup>1</sup> and Shawn D. Askew<sup>2</sup>

<sup>1</sup>Graduate Research Assistant, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA, USA; <sup>2</sup>Professor, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA, USA

**Author for correspondence:** Shawn Askew, Professor, School of Plant and Environmental Sciences, Virginia Tech, 675 Old Glade Road, Blacksburg, VA, 24060

**Email:** saskew@vt.edu

**Running header:** Endothall annual bluegrass

This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is unaltered and is properly cited. The written permission of Cambridge University Press must be obtained for commercial re-use or in order to create a derivative work.

## Abstract

Increasing instances of herbicide-resistant annual bluegrass have limited turf manager's options for chemical control. Endothall inhibits serine threonine protein phosphatase, a novel site of action for warm-season turf, and endothall use in hybrid bermudagrass is not extensively reported. Greenhouse studies were conducted to evaluate herbicide-resistant annual bluegrass response to endothall. Five herbicide-resistant annual bluegrass biotypes were treated with increasing endothall rates and compared to two susceptible populations. One glyphosate-resistant annual bluegrass biotype was 2.3- to 3.3-fold more resistant to endothall depending on trial and susceptible biotype, and all other biotypes were endothall-susceptible. Four field studies were established from 2022-2023 to evaluate the influence of endothall rate and application timing on bermudagrass and manilagrass turf injury and annual bluegrass control. These studies were arranged as a three-by-four factorial with three levels of application timing (fully dormant, 50% green, and 100% green) and four levels of herbicide (endothall applied at 1.12, 1.68, and 2.24 kg ai ha<sup>-1</sup> and trifloxysulfuron applied at 27.8 g ai ha<sup>-1</sup>). Maximum observed turf injury was dependent on endothall rate and timing and was commercially acceptable (<30%) at the low and middle rates when applied to 100% green bermudagrass and all rates when applied to dormant turf. When applied to 50% green turf (mid-transition), endothall unacceptably injured warm-season turf regardless of application rate, but when applied during mid-transition, only the high endothall rate unacceptably injured turf 14 days after treatment. Endothall controlled annual bluegrass more effectively when applied during mid-transition and 100% green turf than fully dormant turf. When applied at 1.68 and 2.24 kg ai ha<sup>-1</sup> to mid-transition and 100% green turf, endothall controlled annual bluegrass 83-95%. Results from these studies indicate endothall selectively controls herbicide-resistant annual bluegrass in warm-season turf but selectivity and performance depend on application timing.

**Nomenclature:** Endothall; trifloxysulfuron; annual bluegrass, *Poa annua* L.; hybrid bermudagrass, *Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burt Davy. 'Tifway 419' and 'Latitude 36'; manilagrass (*Zoysia matrella* L. Merr.) 'Cavalier'

**Keywords:** Turfgrass, herbicide resistance

## Introduction

Annual bluegrass resistance to herbicide is widespread in intensively managed turf systems. Currently, there are 18 uniquely reported cases of herbicide-resistant annual bluegrass in the United States (Heap 2023), and that is likely under-reported based on recent literature (Bowling et al. 2024; Ignes et al. 2023; Rutland et al. 2023). Herbicide-resistant annual bluegrass has proliferated in warm-season turf systems across a variety of herbicide modes of action. A recent survey of Tennessee golf courses identified 21%, 64%, 58%, and 97% of annual bluegrass as resistant to glyphosate, foramsulfuron, prodiamine, and simazine, respectively (Brosnan et al. 2020c). Additionally, 4% of these resistant populations exhibited resistance to multiple herbicide modes of action (Brosnan et al. 2020c). Similar surveys in Mississippi and Texas indicate that annual bluegrass herbicide resistance is widespread across the southern US and that annual bluegrass populations with multiple herbicide resistance are prevalent (Hutto et al. 2004; Singh et al. 2021). In many instances, annual bluegrass evolves resistance to multiple herbicide modes of action when resistant populations are initially managed with a single alternate herbicide (Brosnan et al. 2020a). Furthermore, resistant annual bluegrass populations are more prevalent in warm-season turf systems (Brosnan et al. 2020b; Cross et al. 2015; Isgrigg et al. 2002; Kelly et al. 1999; McElroy et al. 2013; Yu et al. 2018). Due to the widespread nature of herbicide-resistant annual bluegrass, herbicide options with alternative modes of action are needed to sustain effective annual bluegrass control in warm-season turf systems.

Endothall is currently utilized in the US as an aquatic herbicide for control of a variety of weed species (Skogerboe and Getsinger 2002). In US turf, endothall was historically used for semi-selective control of annual bluegrass in cool season turfgrasses. In a creeping bentgrass (*Agrostis stolonifera* L.) fairway, three spring applications of endothall applied at 0.56 kg ha<sup>-1</sup> reduced annual bluegrass coverage by 62% relative to the nontreated (Engel and Aldrich 1960). In Michigan, three biweekly applications of endothall applied at 0.3 and 0.6 kg ai ha<sup>-1</sup> to Kentucky bluegrass (*Poa pratensis* L.) turf reduced annual bluegrass coverage from 50% to 32% and 17%, respectively (Turgeon et al. 1972a). Endothall's effectiveness for annual bluegrass control, however, has been limited by cool-season turf phytotoxicity (Peppers et al. 2021).

Recent literature indicates bermudagrass may be more tolerant than cool-season turf species to endothall applications. Bermudagrass (*Cynodon dactylon* L. Pers.) was 57 and 65 times more tolerant to endothall-contaminated irrigation water compared to that of annual

bluegrass and annual ryegrass, respectively (Koschnick et al. 2005). Additionally, endothall applied at 0.84 and 1.68 kg ai ha<sup>-1</sup> was not significantly injurious to hybrid bermudagrass (*Cynodon transvaalensis* Burt Davy × *dactylon* (L.) Pers.) putting greens when applied during full winter dormancy (Peppers and Askew 2023). To date, no peer-reviewed literature has evaluated endothall for annual bluegrass control in bermudagrass (*Cynodon dactylon* (L.) Pers.) turf. Endothall is a serine/threonine protein phosphatase inhibitor, which is a novel mode of action in turf systems (Bajsa et al. 2012; Tresch et al. 2011). Previous literature indicates that herbicides with novel modes of action can effectively control herbicide-resistant annual bluegrass populations (Brosnan et al. 2017). Endothall-resistant annual bluegrass has already been reported in Australia. Barua et al. (2020) found all annual bluegrass populations screened in Australia were resistant to endothall. However, in this study, the susceptible comparison was controlled 50% by a single application of endothall applied at 0.13 kg ai ha<sup>-1</sup>, which is a much lower rate than has sub-lethally suppressed annual bluegrass in other studies (Engel and Aldrich 1960; Peppers et al. 2021; Turgeon et al. 1972a). The now-lapsed US federal label for terrestrial uses of endothall (EPA 2005) indicates a maximum terrestrial use rate of 2.24 kg ai ha<sup>-1</sup>, which is approximately 10 times higher than the currently labeled rate in Australia (Anonymous 2020). Based on previous literature, we surmise that endothall rates for turfgrass use currently in Australia and formerly in the US were limited due to concerns of injury to cool-season turf and the product's historical market value. In warm-season turf, especially during dormancy, the potential market value for endothall has likely increased due to developed annual bluegrass resistance to a wide range of formerly viable herbicides. Thus, we hypothesized that above-Australian-label endothall dosages may acceptably control herbicide-resistant annual bluegrass with acceptable phytotoxicity to dormant, semidormant, or actively growing bermudagrass turf.

## **Materials and Methods**

### ***Herbicide-resistant annual bluegrass response to endothall***

Two greenhouse studies were conducted at the Glade Road Research Facility in Blacksburg, VA to evaluate herbicide-resistant annual bluegrass response to endothall. Both trials were arranged as randomized complete block designs with 42 treatments and four blocks. Treatments were arranged as a seven-by-eight factorial with seven levels of annual bluegrass populations and eight levels of herbicide treatment. The six annual bluegrass populations consisted of two known

susceptible populations (S1 and S2), two ALS-inhibitor-resistant populations (ALS1 and ALS2), two glyphosate-resistant populations (EPSP1 and EPSP2) as well as a population that exhibits resistance to multiple herbicide modes of action (MR). The multiple-resistant annual bluegrass population was putatively resistant to EPSP, PSII, and HPPD-inhibiting herbicides. All annual bluegrass populations were collected throughout the state of Virginia and screened for herbicide resistance to a variety of modes of action. The causal mechanisms of resistance are more thoroughly presented in Table 1. The six herbicide levels include five endothall rates (0.5, 1, 2, 4, and 8 kg ai ha<sup>-1</sup>) and a nontreated comparison for every annual bluegrass population.

All populations were seeded into 45 by 30 cm flats containing potting soil (Pro-Mix BXM General Purpose Growing Medium, Premier Horticulture Inc., Quakertown, PA). After annual bluegrass plants reached the two-tiller growth stage, the herbicide-resistant annual bluegrass populations were treated with appropriate herbicides to ensure homogeneity of resistant annual bluegrass plants. Populations ALS1, ALS2, and MR were treated with trifloxysulfuron (Monument® Syngenta Crop Protection LLC) applied at 27.8 g ai ha<sup>-1</sup> plus non-ionic surfactant (Induce, Helena®, Chemical Company, Collierville, TN) applied at 0.25% v/v, populations EPSP1 and EPSP2 were treated with glyphosate (Roundup Pro Concentrate; Monsanto Company) applied at 1.12 kg ai ha<sup>-1</sup>. Surviving plants were selected and transplanted into 7.6 by 7.6 by 6.4 cm pots containing a 2:1 sand-to-native soil mixture. The native soil utilized in this study was a Groseclose-Urban land complex loam (clayey, mixed, mesic Typic Hapludults) with a pH of 6.0 and 3.1% organic matter. All plants were fertilized with 25 kg N ha<sup>-1</sup> (19-6-12; Sta-Green Indoor and Outdoor All-Purpose Food Fertilizer, Gro Tec, Inc.) once approximately 1 wk after germination to maintain proper plant growth. Irrigation was supplied twice daily to prevent plant wilt. Mercury vapor lamps provided 430 μmol m<sup>-2</sup> sec<sup>-1</sup> of photosynthetically active radiation as supplemental lighting and was set to a 14-hr daylength throughout the studies. Greenhouse day/night temperatures were maintained at 29/20 C. All treatments were applied using a CO<sub>2</sub>-pressurized backpack sprayer calibrated to deliver 375 L ha<sup>-1</sup> at 330 kPa fitted with TTI11004 nozzles (TeeJet Technologies, Springfield, IL). Following applications, all pots were allowed to dry for 4 to 6 hr before irrigation being applied. Annual bluegrass plants had approximately 5-8 tillers at application. At the conclusion of the trial, 28 d after application, annual bluegrass above-ground biomass was collected and dried at 50 C for 72 hr before weighing. Annual bluegrass control data were extrapolated from the above-ground

biomass data by comparing all treated plants to the nontreated comparison within a given biotype and replication. The endothall rate required to control annual bluegrass 50 and 90% ( $C_{50}$  and  $C_{90}$ , respectively) was modeled across all doses in each replicate for a given biotype using a three-parameter sigmoidal model with equation 1:

$$\% = a / (1 + \exp [(-(-(x - x_0) / b))]) \quad [1]$$

where % represents percent annual bluegrass control,  $x$  represents the endothall rate, and  $a$ ,  $b$ , and  $x_0$  represent regression parameters calculated using PROC NLIN in SAS 9.4 (SAS Institute Inc, Cary, NC). The resulting  $C_{50}$  and  $C_{90}$  data were subjected to ANOVA using PROC GLM in SAS 9.4 with sums of squares partitioned to reflect replicate, trial, and biotype and means were separated with Fisher's protected LSD test at  $\alpha = 0.05$ .

### ***Field evaluation of annual bluegrass control and turf tolerance to endothall***

Four field studies were conducted in Blacksburg, VA (37.22°N, 80.41°W) between February 2022 and June 2023 to evaluate annual bluegrass and warm season turf response to endothall. Two field studies, one in 2022 (TRC1) and one in 2023 (TRC2), were conducted on a 'Tifway 419' hybrid bermudagrass research fairway maintained at 1.5 cm in height at the Virginia Tech Turfgrass Research Center. Two additional field studies were conducted at the Glade Road Research Facility in 2022 (GRRF1) on 'Cavalier' manilagrass and in 2023 (GRRF2) on a 'Latitude 36' hybrid bermudagrass (Table 2). Both locations at the Glade Road Research Facility were research fairways maintained at 1.3 cm height throughout the study. All field trials were arranged as randomized complete block designs with 13 treatments and four blocks. Treatments were arranged as a three-by-four factorial with three levels of application timing (fully dormant, 50% visible green turf coverage, and 100% visible green turf coverage) and four levels of herbicide treatment. The four levels of herbicide treatment were endothall (Teton; United Phosphorus, Inc.) applied at 1.12, 1.68, and 2.24 kg ai ha<sup>-1</sup> and trifloxysulfuron (Monument® Syngenta Crop Protection LLC) applied at 27.8 g ai ha<sup>-1</sup>. A nontreated comparison was included in all trials. Applications were applied via a CO<sub>2</sub>-pressurized sprayer fitted with flat fan 6503 nozzles (TeeJet Technologies, Springfield, IL) and calibrated to deliver 375 L ha<sup>-1</sup> at 330 kPa. Plots measured 0.9 × 1.8 m in size. Fully dormant, 50% green, and 100% green applications were made on April 5, April 25, and May 12 in 2022, respectively and March 16, April 26, and May 10 in 2023, respectively. Annual bluegrass had 80-120 tillers at the earliest

application timing and greater than 150 tillers at the mid-transition and fully green turf application timings.

Annual bluegrass control and turf green coverage were visually evaluated throughout the study as a percentage in which 0% equals no annual bluegrass control or no visible green turf, 100% equals complete annual bluegrass control or complete green turf coverage, and 80% annual bluegrass control was considered commercially acceptable. Turf injury was extrapolated from green coverage assessment by calculating the percent green coverage reduction in treated plots compared to the nontreated check. At the conclusion of the studies (~June 1), annual bluegrass density was measured via line-intersect grids that included 135 intersects at 5.5 cm increments within the treated portion of each plot. Final assessments of weed control were extrapolated from the line-intersect counts by comparing weed coverage in the nontreated check in each replication versus the coverage in each treated plot. Data were subjected to ANOVA with sums of squares partitioned to reflect replicate and trial as random variables; herbicide, application timing, and herbicide by application timing as fixed effects; and all possible interactions of trial with fixed effects. Terms that included trial interactions were tested with the mean square of residual error. The terms herbicide, application timing, and herbicide by application timing were tested with the mean square associated with each terms' interaction with trial as described by McIntosh (1983). Main effects or interactions were pooled over trial only if trial interactions were insignificant. Appropriate means were separated with Fisher's protected LSD test at  $\alpha = 0.05$ .

## **Results and Discussion**

### ***Herbicide-resistant annual bluegrass response to endothall***

The trial by biotype interaction was significant for annual bluegrass  $C_{50}$  and  $C_{90}$  ( $P = 0.0077$  and  $0.0008$ , respectively); therefore, results are presented separately by trial. The trial interaction was likely due to differences in endothall efficacy between the two trials with respect to the S2 biotype, as this is the only biotype that changed the mean rank between trials for either  $C_{50}$  or  $C_{90}$  (Table 3). Although differences between biotypes other than S2 were similar in both trials, 26 to 53% more endothall was required to control annual bluegrass in trial 1 compared to trial 2, for all biotypes except S2 and EPSP2. The average amount of endothall needed to control susceptible annual bluegrass 90% was  $1.6 \text{ kg ha}^{-1}$  (Table 3). It was surmised that light intensity under greenhouse conditions were less than full sunlight, and this may have reduced endothall

performance given the herbicide's mode of action (Dodge 1982; Mayasich et al. 1986). In trial 2, plants were moved outdoors under full sunlight at treatment time and remained for 3 d following treatment, and endothall more effectively controlled all biotypes except S2 and EPSP2. Similar differences occurred between field and greenhouse performance of endothall when 4.5 kg endothall ha<sup>-1</sup> was required to reduce annual bluegrass foliar length in greenhouse studies in 1972 (Turgeon et al. 1972a).

Only EPSP2 required more endothall to achieve C<sub>50</sub> than both susceptible populations in both trials (Table 3). Interestingly, EPSP2 and EPSP1 each had a Pro-106-Ala amino acid substitution on 5-enolpyruvylshikimate3-phosphate synthase (EPSPS), but only EPSP2 exhibited endothall resistance. This disparate endothall response in similar EPSP-resistant biotypes that had not been previously exposed to endothall indicates the Pro-106-Ala substitution does not confer endothall resistance. The endothall resistance mechanism in EPSP2 is unknown. Previous research indicates annual bluegrass populations with nontarget site resistance can be resistant to novel modes of action (Brosnan et al. 2017), but there also may be other mechanisms that could explain the resistance of the EPSP2 population to endothall. It should be noted that ALS1 did not have an observed target site mutation and was not resistant to endothall. Nontarget site resistance is most observed in row crop systems but has been reported in annual bluegrass from turf systems. In an Alabama population, annual bluegrass resistance to PSII-inhibiting herbicides was conferred via reduced herbicide absorption and translocation (Syvanteck et al. 2016). An annual bluegrass population in Tennessee resistant to multiple herbicide modes of action exhibited increased levels of transporter and metabolic enzymes (Brosnan et al. 2019). ALS1 and EPSP2 may exhibit different resistance mechanisms, leaving open the possibility of a nontarget site mechanism of resistance to endothall in EPSP2.

### ***Field evaluation of annual bluegrass control and turf tolerance to endothall***

Over 90% of bermudagrass winter damage occurred at GRRF2. Therefore, turf injury data were not collected at this location. Due to differences in herbicide speed of activity between endothall and trifloxysulfuron, turf injury data were converted to the maximum observed injury over the assessment period. Maximum turf injury did not differ due to trial ( $P \geq 0.1854$ ). However, the interaction of herbicide by application timing was significant ( $P = 0.0002$ ). Therefore, data are pooled over trials, and the herbicide by application timing interaction is shown (Table 4). Herbicides did not unacceptably ( $> 30\%$ ) injure fully-dormant turf, and only



endothall applied at 2.24 kg ai ha<sup>-1</sup> injured actively growing turf > 30%. In general, semi-dormant turf was more sensitive to endothall than fully dormant or green turf. These results align with previous literature, as warm-season turf is generally injured more by herbicides applied during post-dormancy transition (Dernoeden 1994; Johnson 1976; Peppers and Askew 2023; Reed and McCullough 2014; Reed et al. 2015). Turf injury during post-dormancy transition generally increased with endothall rate to as much as 60% (Table 4).

Despite unacceptable maximum injury at any endothall rate during post-dormancy transition and the highest rate on green turf, turf recovered to acceptable levels by 14 d after treatment (DAT) for all treatments except the highest endothall rate applied to transitioning turf (Table 4). By 28 DAT, treatments did not injure turf more than 13% (data not shown). Hybrid bermudagrass putting greens responded similarly to endothall application timings and exhibited rapid recovery whenever bermudagrass was injured in other studies (Peppers and Askew 2023). Additionally, warm-season turf demonstrated endothall tolerance relative to historical reports of cool-season turf response (Engel and Aldrich 1960; Peppers et al. 2021; Turgeon et al. 1972a), which is consistent with studies that evaluated turf tolerance to endothall-contaminated irrigation water (Koschnick et al. 2005).

Although the three-way interaction of trial by herbicide by application timing on annual bluegrass control at the conclusion of the trial was significant ( $P = 0.0495$ ), the low F value for the interaction (2.6) compared to the herbicide by application timing main effect (F value=11.4,  $P=0.002$ ) suggests that the interaction explains a small amount of the variance. The three-way interaction was likely significant due to variability in annual bluegrass control with trifloxysulfuron in relation to endothall treatments. For example, at GRRF1, trifloxysulfuron applied during mid-transition controlled annual bluegrass 93% and more than all endothall treatments. At GRRF2, trifloxysulfuron applied during mid-transition controlled annual bluegrass 72% and less than all endothall treatments (data not shown). Additionally, when trifloxysulfuron treatments were removed from the analysis, the trial interaction was no longer significant ( $P = 0.7041$ ) indicating trifloxysulfuron was the primary cause of the three-way interaction. For these reasons, data were pooled to show the herbicide by application timing interaction, which was also significant ( $P < 0.0001$ ) for clarity and brevity (Table 4).

Endothall applied to fully dormant turf controlled annual bluegrass 49 to 72% depending on rate and less at any given rate than when applied during or after post-dormancy transition

(Table 4). This reduction in endothall efficacy when applied during full dormancy may be attributed to a variety of factors. Temperature and sunlight intensity were inherently lower during the fully dormant applications. Average temperatures at the time of the fully dormant applications were 8 to 9 C, and average temperatures at the time of both later applications were 18 to 23 C (data not shown). Previous research indicates that herbicide activity is positively correlated to temperature and light intensity (Dodge 1982; Kells et al. 1984; Mayasich et al. 1986; McWhorter and Azlin 1978; Wills and McWhorter 1981). Endothall applied at 1.68 and 2.24 kg ai ha<sup>-1</sup> to post-dormant turf controlled annual bluegrass 88-95% and equivalent to or better than trifloxysulfuron (Table 4).

These results indicate that endothall controls annual bluegrass acceptably at any of the rates tested when applied to fully green turf and injures bermudagrass not more than 21% at rates of 1.68 kg ha<sup>-1</sup> or lower. Endothall does not injure fully dormant turf, but single treatments are unlikely to acceptably control annual bluegrass at this timing. Endothall should not be applied to bermudagrass during post-dormancy transition unless severe but transient injury can be tolerated. To avoid turf injury, it may be possible to make multiple applications of endothall or include other herbicide admixtures. Previous research indicates that multiple endothall applications at lower rates control annual bluegrass more effectively than single endothall applications at higher rates (Turgeon et al. 1972a). All annual bluegrass evaluated in this study had greater than 80 tillers at the time of application and endothall may more effectively control relatively less mature annual bluegrass, as weed growth stage regularly affects herbicide activity (Bellinder et al. 2003; Busey 2004; Peppers et al. 2024; Steckel et al. 1997). Additionally, surfactant was not included with endothall in these studies. Adjuvants such as nonionic surfactants or crop oil concentrates can increase herbicide efficacy (Norsworthy and Grey 2004; Reichers et al. 1994; Sherrick et al. 1986) and may increase annual bluegrass control by a given endothall rate.

### **Practical Implications**

Results from these studies indicate endothall can provide selective annual bluegrass control in warm-season turf species, even on herbicide-resistant annual bluegrass populations if mid-transition treatments are avoided. Endothall applied at rates of 1.12 and 1.68 kg ai ha<sup>-1</sup> controls annual bluegrass when applied to fully green turf, however, annual bluegrass is unlikely to be controlled more than 75% when single treatments are applied during dormancy. Turf was most tolerant to endothall when applied during full dormancy. To improve weed control while

capitalizing on turf safety during dormancy, future research should evaluate herbicide and surfactant admixtures with endothall applied at earlier application timings. To reduce potential for phytotoxicity on fully green turf, post application irrigation should be investigated as a means to reduce foliar injury since endothall is primarily translocated acropetally (Turgeon et al. 1972b). Sequential herbicide applications at lower rates are commonly more effective than single applications at higher rates (Willis et al. 2006) and should be investigated for potential to improve annual bluegrass control in dormant or green turf and reduce phytotoxicity in green turf.

### **Acknowledgments**

The authors would like to thank Mr. John Hinson for maintaining turf plots at the Virginia Tech Turfgrass Research Center for the duration of these studies.

### **Funding**

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

### **Conflict of Interest**

The authors declare no conflict of interest.

### **References**

- Anonymous (2020) PoaChek<sup>®</sup> specimen label. Colin Campbell Chemicals Pty Ltd. Wetherill Parl New South Wales, Australia.
- Bajsa J, Pan Z, Dayan FE, Owens DK, Duke SO (2012) Validation of serine/threonine protein phosphatase as the herbicide target site of endothall. *Pestic Biochem Physiol* 102:38–44
- Barua R, Bousalis P, Malone J, Gill G, Preston C (2020) Incidence of multiple herbicide resistance in annual bluegrass (*Poa annua*) across southeastern Australia. *Weed Sci* 68:340–347
- Bellinder RR, Arsenovic M, Shah DA, Rauch BJ (2003) Effect of weed growth stage and adjuvant on the efficacy of fomesafen and bentazon. *Weed Sci* 51:1016–1021
- Bowling RG, McCurdy JD, de Castro EB, Patton AJ, Brosnan JT, Askew SD, Breeden GK, Elmore MT, Gannon TW, Gonçalves CG, Kaminski JE, Kowalewski AR, Liu W, Mattox CM, McCarty LB, McCullough PE, McElroy JS, McKeithen C, Osburn AW, Rogers RR, Rutland CA, Tang K, Taylor JW, Unruh JB, Vargas JJ, Bagavathiannan MB (2024) Multi-

state survey to identify suspected resistance to four herbicides and one plant growth regulator in *Poa annua*. *Crop Forage and Turf Manage* e10:20300.

- Busey P (2004) Goosegrass (*Eleusine indica*) control with foramsulfuron in bermudagrass (*Cynodon* spp.) turf. *Weed Technol* 18:634–640
- Brosnan JT, Elmore ME, Bagavathiannan MV (2020a) Herbicide-resistant weeds in turfgrass: current status and emerging threats. *Weed Technol* 34:424–430
- Brosnan JT, Laforest M, Soufiane B, Boggess SL, Trigiano RN (2019) Target and non-target site resistance mechanisms in a *Poa annua* biotype from Tennessee. Page 10 in *Proceedings of the Resistance '19 Conference*, September 16–18, Rothamsted Research, Harpenden, UK. <https://www.rothamsted.ac.uk/resistance19#PROGRAMME-1>
- Brosnan JT, Vargas JJ, Spesard B, Netzband D, Zobel JM, Chen J, Patterson EL (2020b) Annual bluegrass (*Poa annua*) resistance to indaziflam applied early-postemergence. *Pest Manag Sci* 76: 2049–2057
- Brosnan JT, Vargas JJ, Breeden GK, Zobel JM (2020c) Herbicide resistance in annual bluegrass on Tennessee golf courses. *Crop Forage & Turf Manage* 6:e20050
- Brosnan JT, Vargas JJ, Breeden GK, Boggess SL, Staton MA, Wadl PA, Trigiano RN (2017) Controlling herbicide-resistant annual bluegrass (*Poa annua*) phenotypes with methiozolin. *Weed Technol* 31:470–476
- Cross RB, McCarty LB, Tharayil N, McElroy JS, Chen S, McCullough PE, Powell BA, Bridges Jr WC (2015) A Pro106 to Ala substitution is associated with resistance to glyphosate in annual bluegrass (*Poa annua*). *Weed Sci* 63:613–622
- Dernoeden PH (1994) Perennial ryegrass control in bermudagrass and zoysiagrass. *J Turfgrass Manag* 1:31–47
- Dodge AD (1982) The role of light and oxygen in the action of photosynthesis inhibitor herbicides. p 57–77 in DE Moreland, JB St. John, and FD Hess, eds. *Biochemical responses induced by herbicides*. Am Chem Soc Symp Ser No 181
- Engel RE, Aldrich RJ (1960) Reduction of annual bluegrass, *Poa annua*, in bentgrass turf by the use of chemicals. *Weeds*, 8, 26–28.
- Ignes M, McCurdy JD, McElroy JS, Castro EB, Ferguson JC, Meredith AN, Rutland CA, Stewart BR, Tseng TP (2023) Target- and non-target site mechanisms of pronamide

- resistance in annual bluegrass (*Poa annua*) populations from Mississippi golf courses. *Weed Sci* 71:206–216
- Isgrigg III J, Yelverton FH, Brownie C, Warren Jr LS (2002) Dinitroaniline resistant annual bluegrass in North Carolina. *Weed Sci* 50:86–90
- Johnson BJ (1976) Bermudagrass tolerance to consecutive butralin and oxadiazon treatments. *Weed Sci* 24:302–305
- Kells JJ, Meggitt WF, Penner D (1984) Absorption, translocation, and activity of fluazifop-butyl as influenced by plant growth stage and environment. *Weed Sci* 32:143–149
- Koschnick TJ, Haller WT, Fox AM (2005) Turf and ornamental plant tolerances to endothall in irrigation water II. turf species. *Hort Tech* 15:324–329
- Mayasich JM, Karlander EP, Terlizzi Jr. DE (1986) Growth responses of *Nannochlorisoculata* droop and *Phaeodactylum tricornutum* bohlin to the herbicide atrazine as influenced by light intensity and temperature. *Aquat Toxicol* 8:175–184
- McElroy JS, Flessner ML, Wang Z, Dane F, Walker RH, Wehtje G (2013) A Trp<sub>574</sub> to Leu amino acid substitution in the ALS gene of annual bluegrass (*Poa annua*) is associated with resistance to ALS-inhibiting herbicides. *Weed Sci* 61:21–25
- McIntosh MS (1983) Analysis of combined experiments. *Agron. J.* 75:153–155
- McWhorter CG, Azlin WR (1978) Effects of environment on the toxicity of glyphosate to johnsongrass (*Sorghum halepense*) and soybeans (*Glycine max*). *Weed Sci* 26:605–608
- Norsworthy JK, Grey TL (2004) Addition of nonionic surfactant to glyphosate plus chlorimuron. *Weed Technol* 18:588–593
- Peppers JM, Askew SD (2023) Herbicide effects on dormant and post-dormant hybrid bermudagrass putting green turf. *Weed Technol* 37:522–529
- Peppers JM, Brewer JR, Askew SD (2021) Plant growth regulator and low-dose herbicide programs for annual bluegrass seedhead suppression in fairway and athletic-height turf. *Agron J* 113:3800–3807
- Reed TV, McCullough PE (2014) Tolerance of five warm-season turfgrasses to flumioxazin. *Weed Technol* 28:340–350
- Reed TV, McCullough PE, Grey T, Czarnota MA, Vencill WK, Waltz FC (2015) Flumioxazin tank-mixtures with six herbicides for annual bluegrass (*Poa annua*) control in bermudagrass. *Weed Technol* 29:561–569

- Riechers DE, Wax LM, Liebl RA, Bush DR (1994) Surfactant increased glyphosate uptake into plasma membrane vesicles isolated from common lambsquarters leaves. *Plant Physiol* 105:1419–1425
- Sherrick SL, Holt HA, Hess FD (1986) Effects of adjuvants and environment during plant development on glyphosate absorption and translocation in field bindweed (*Convolvulus arvensis*). *Weed Sci* 34: 811–816
- Singh V, Reis FC, Reynolds C, Elmore ME, Bagavathiannan MK (2021) Cross and multiple herbicide resistance in annual bluegrass (*Poa annua*) populations from eastern Texas golf courses. *Pest Manage Sci* 77: 1903–1914
- Skogerboe JG, Getsinger KD (2002) Endothall species selectivity evaluation: northern latitude aquatic plant community. *J Aquatic Plnt Manage* 40:1–5
- Steckel GJ, Wax LM, Simmons FW, Phillips WH (1997) Glufosinate efficacy on annual weeds is influenced by rate and growth stage. *Weed Technol* 11:484–488
- Syvantek AW, Aldahir P, Chen S, Flessner ML, McCullough PE, Sidhu SS, McElroy JS (2016) Target and non-target resistance mechanisms induce annual bluegrass (*Poa annua*) resistance to atrazine, amicarbazone, and diuron. *Weed Technol* 30:773–782
- Trappe JM, Karcher DE, Richardson MD, Patton AJ (2011) Shade and traffic tolerance varies for bermudagrass and zoysiagrass cultivars. *Crop Sci* 51:870–877
- Tresch S, Schmotz J, Grossman K (2011) Probing mode of action in plant cell cycle by the herbicide endothall, a protein phosphatase inhibitor. *Pestic Biochem Physiol* 99:86–95
- Turgeon AJ, Meggitt WF, Penner D (1972a) Role of endothall in the control of annual bluegrass in turf. *Weed Sci* 20:562–565
- Turgeon AJ, Penner D, Meggitt WF (1972b) Selectivity of endothall in turf. *Weed Sci* 20:557–561
- Willis JB, Beam JB, Barker WL, Askew SD (2006) Weed control options in spring-seeded tall fescue (*Festuca arundinacea*). *Weed Technol* 20:1040–1046
- Wills GD, McWhorter CG (1981) Effect of environment on the translocation and toxicity of acifluorfen to showy crotonia (*Crotalaria spectabilis*). *Weed Sci* 29:397–401
- Yu J, McCullough P, Czarnota M (2018) Annual bluegrass (*Poa annua*) biotypes exhibit differential levels of susceptibility and biochemical responses to protoporphyrinogen oxidase inhibitors. *Weed Sci* 66:574–580

Table 1. Herbicide-resistant annual bluegrass biotypes screened for endothall resistance.

Biotype	Location collected	Growing condition	Putative resistance	Target site mutation
S1	Charlottesville, VA	Sports field	none	none detected
S2	Keswick, VA	Golf putting green	none	none detected
ALS1	Lansdowne, VA	Golf fairway	ALS	none detected
ALS2	Lansdowne, VA	Golf tee box	ALS	Trp574-Leu <sup>a</sup>
EPSP1	Chesterfield, VA	Golf fairway	EPSP	Pro106-Ala <sup>b</sup>
EPSP2	Keswick, VA	Golf fairway	EPSP	Pro106-Ala
Multi	Laurel, VA	Sports field	ALS, PSII, HPPD, mitosis inhibitor	none detected

<sup>a</sup>Annual bluegrass ALS-herbicide resistance due to a Trp574-Leu mutation was first reported by McElroy et al. (2013).

<sup>b</sup>Annual bluegrass glyphosate resistance due to a Pro106-Ala mutation was first reported by Cross et al. (2015).

Table 2. Trial locations and application timings of field-applied endothall.

Year	Trial	Species	Cultivar	Application timing		
				Fully dormant	Mid-transition	100% green
2022	TRC1	Hybrid bermudagrass	'Tifway 419'	April 5	April 25	May 12
2022	GRRF1	Manilagrass	'Companion'	April 5	April 25	May 12
2023	GRRF2	Hybrid bermudagrass	'Latitude 36'	March 16	April 26	May 10
2023	TRC2	Hybrid bermudagrass	'Tifway 419'	March 16	April 26	May 10



Table 3. Influence of biotype on endothall rate required to control annual bluegrass 50 and 90% ( $C_{50}$  and  $C_{90}$ , respectively).

Biotype	$C_{50}$				$C_{90}$			
	Trial 1 <sup>a</sup>		Trial 2		Trial 1		Trial 2	
	g ai ha <sup>-1</sup>							
S1 <sup>b</sup>	2347	Bc <sup>c</sup>	1595	b	5392	ab	2649	b
S2	1300	c	1675	b	1882	c	2657	b
ALS1	1810	bc	1184	b	4334	abc	1842	b
ALS2	1660	bc	1310	b	5209	ab	2530	b
EPSP1	2602	b	1818	b	3589	bc	2711	b
EPSP2	4242	a	3817	a	6566	a	6306	a
Multi	2265	bc	1477	b	3481	bc	2314	b

<sup>a</sup>Trial 1 remained under greenhouse supplemental lighting conditions throughout the duration of the study. However, annual bluegrass plants in trial 2 were moved outdoors for three d immediately following application to mimic field light intensity.

<sup>b</sup>Biotypes were tested for putative resistance in previous studies and only survivors of prescreened populations were used in the current study. Abbreviations are S1 and S2 for susceptible populations, ALS1 and ALS2 for biotypes resistant to acetolactate synthase inhibiting herbicides, EPSP1 and EPSP2 for biotypes resistant to glyphosate, and “multi” for a biotype with confirmed resistance to four herbicide modes of action.

<sup>c</sup>Letters following means indicate significant differences, according to Fisher’s protected LSD test ( $\alpha = 0.05$ ), within a given column.

Table 4. Maximum observed turfgrass injury and end-of-season annual bluegrass control as influenced by herbicide at three application timings (dormant, mid-transition, and 100% green turf).

	Maximum observed turf injury								Annual bluegrass control <sup>a</sup>						
	Rate	Dormant		Mid-transition		100% green		LSD <sup>b</sup>	Dormant		Mid-transition		100% green		
Herbicide	g ai ha <sup>-1</sup>	%													
Endothall	1120	6	b <sup>c</sup>	32	b	13	bc	11	49	b	77	b	83	c	10
Endothall	1680	28	a	43	b	21	b	17	71	a	88	a	90	ab	9
Endothall	2240	24	ab	60	a	35	a	19	72	a	91	a	95	a	10
Trifloxysulfuron <sup>d</sup>	27.8	18	ab	11	c	3	c	12	83	a	86	a	89	bc	9

<sup>a</sup>Annual bluegrass control was derived via line-intersect counts that included 135 intersects at 5.5 cm increments within the treated portion of each plot. Final assessments of weed control were extrapolated from the line-intersect counts by comparing weed coverage in the nontreated check in each replication versus the coverage in each treated plot.

<sup>b</sup>LSD denotes statistical significance, according to Fisher's protected LSD test ( $\alpha=0.05$ ), within rows for a given rating metric.

<sup>c</sup>Letters following means indicate significant differences, according to Fisher's protected LSD test ( $\alpha=0.05$ ), within a given column.

<sup>d</sup>Nonionic surfactant was included at 0.25% v/v in all trifloxysulfuron treatments.