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Short title: Japanese stiltgrass control

Assessing Herbicides for Japanese Stiltgrass (*Microstegium vimineum*) Control in Cool-Season Turfgrass

Navdeep Godara¹, John R. Brewer², and Shawn D. Askew³

¹Graduate Assistant (ORCID 0000-0002-1452-5225), School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University; ²Graduate Assistant (ORCID 0000-0001-6492-1296), School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University; and ³Professor (ORCID 0000-0002-9515-9502), School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA

Author for correspondence: Shawn D Askew, Professor, School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University, 675 Old Glade Road, Blacksburg, VA 24061 **E-mail:** saskew@vt.edu

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Abstract

Japanese stiltgrass is one of the most troublesome invasive weed species in the eastern United States. Strategies for Japanese stiltgrass control in managed lawns are limited since most previous research was conducted in forest understories or golf course natural areas. Eight field experiments were conducted in Virginia from 2014 to 2019 to evaluate the response of Kentucky bluegrass, tall fescue, and Japanese stiltgrass to selective herbicides traditionally marketed for cool-season turfgrass. Only mesotrione-containing treatments transiently injured tall fescue 15 to 25% at 2 wk after treatment (WAT). Fenoxaprop at 35 g ha⁻¹ or higher rates controlled Japanese stiltgrass \geq 90%, reduced relative cover to $<$ 15% compared to nontreated and shoot density to \leq 6 shoots m⁻², respectively, at 8 WAT. Sequential application of topramezone at 3-wk intervals at 27 g ha⁻¹, single application of topramezone at 54 g ha⁻¹ alone or with triclopyr controlled Japanese stiltgrass \geq 80% and reduced relative weed cover and shoot density to \leq 22% and $<$ 35 shoots m⁻², respectively. Fenoxaprop at 0.25 of the labeled rate for annual grass control in lawns and topramezone-based programs selectively controlled Japanese stiltgrass without injuring tall fescue. Fluazifop at 53 g ha⁻¹ injured Kentucky bluegrass 25% and reduced digitally assessed turf cover by 20% at 4 WAT, but turfgrass recovered by 6 WAT. Reduced rates of fluazifop controlled Japanese stiltgrass 85% and reduced weed shoot density to $<$ 20 shoots m⁻² and relative cover to $<$ 20% at 8 WAT. A premix of dicamba, fenoxaprop, and fluroxypyr did not injure Kentucky bluegrass but controlled Japanese stiltgrass \geq 92% and reduced the relative weed cover and shoot density to \leq 7% and \leq 5 shoots m⁻², respectively, at 8 WAT. Our research provides herbicide options to practitioners to manage Japanese stiltgrass in Kentucky bluegrass and tall fescue lawns.

Nomenclature: Dicamba; fenoxaprop; fluazifop; fluroxypyr; mesotrione; topramezone; triclopyr; Japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus; Kentucky bluegrass, *Poa pratensis* L.; tall fescue, *Lolium arundinaceum* (Schreb.) Darbysh.

Key words: Invasive weed, postemergence herbicides, turfgrass tolerance, weed control

Introduction

Japanese stiltgrass is characterized as an annual, C₄ invasive grassy weed species, colonizing areas from New York to Puerto Rico (Barden 1987, Fairbrothers and Gray 1972). Japanese stiltgrass has the adaptability to invade disturbed and undisturbed areas, including riverbanks, wetlands, woodlands, roadside ditches, utility corridors, as well as landscape bedding and turfgrass (Derr 1999; Fairbrothers and Gray 1972; Redman 1995; Swearingen and Adams 2008). One of the main reasons that Japanese stiltgrass has gained such broad invasion success is derived from its ability to grow under light intensity ranging from full sun to almost fully shaded areas (Horton and Neufeld 1998). The shade tolerance of Japanese stiltgrass distinguishes it from most other C₄ grasses, such as smooth crabgrass [*Digitaria ischaemum* (Schreb.) ex Muhl.] (Brown 1977; Winter et al. 1982).

Winter et al. (1982) demonstrated that Japanese stiltgrass can maintain dry matter production at 18% of full sunlight and grow equivalent to plants maintained under full sunlight. Japanese stiltgrass can grow and produce viable seeds even at 2 to 8% of full sunlight (Cheplick 2005). Japanese stiltgrass exhibits phenotypic plasticity, can grow without sufficient nutrients under challenging environmental conditions, and inhibits the growth of native species (Swearingen and Adams 2008; Ziska et al. 2015). Japanese stiltgrass can also form large sprawling mats that can grow from 0.6 to 1 m tall, which can shade out other native plants and produce up to of 1000 seeds per plant (Swearingen and Adams 2008; Miller and Matlack 2010). These traits allow Japanese stiltgrass to outcompete most understory native species, making it a serious threat to native plant communities and ecosystem function (Culpepper et al. 2018; Miller 2003).

Japanese stiltgrass management is challenging due to its ability to spread rapidly and produce seeds that can remain viable for up to 5 yr (Swearingen and Adams 2008; Tu 2000). Japanese stiltgrass control can be achieved by manual or mechanical measures but herbicides are recommended for controlling large infestations (Shelton 2012; Tu 2000). Previous research assessed several preemergence (PRE) and postemergence (POST) herbicides to control Japanese stiltgrass in forest environments (Flory 2010; Gover et al. 2003; Judge et al. 2005a; Ward and Mervosh 2012), but only a few extension publications have reported selective Japanese stiltgrass control in cool-season turfgrass despite increasing infestations (Nitzsche and Rector 2023).

Judge et al. (2005b) demonstrated that PRE herbicides labeled for large crabgrass [*Digitaria sanguinalis* (L.) Scop.] control in cool-season turfgrass may also provide PRE control of Japanese stiltgrass. These included dithiopyr, prodiamine, trifluralin, oxadiazon, isoxaben, and pendimethalin. Selective and non-selective herbicides applied POST have also been effective in Japanese stiltgrass control including sethoxydim, clethodim, imazapic, fenoxaprop, MSMA, fluazifop, glyphosate, and glufosinate (Gover et al. 2003, Judge et al. 2005b, Weaver et al. 2020), but only fenoxaprop and fluazifop are registered for selective weed control in cool-season turfgrass lawns (Anonymous 2022a). In order to expand Japanese stiltgrass control options in managed cool-season turf, more information is needed on its response to traditional POST lawn herbicides such as topramezone, mesotrione, quinclorac, and combinations of topramezone plus triclopyr and mesotrione plus triclopyr. We hypothesized that topramezone, mesotrione, or quinclorac-based programs will control Japanese stiltgrass equal to or greater than fenoxaprop and fluazifop treatments. The objectives of this study were to assess the effectiveness of different herbicides for Japanese stiltgrass control in tall fescue lawns and to assess selective herbicides to control Japanese stiltgrass in Kentucky bluegrass.

Materials and Methods

Initial Screen of POST Herbicides for Japanese Stiltgrass Control in Tall Fescue. Four field experiments were conducted from 2014 to 2019 to evaluate the tolerance of tall fescue to multiple POST herbicides and their efficacy in controlling Japanese stiltgrass in lawns (Table 1). The study utilized a randomized complete block design with three replications and four temporal runs across two locations. The response of tall fescue to herbicide treatments was assessed in all four studies, whereas Japanese stiltgrass control was evaluated in two of these studies (Table 1). Each plot measured 1.8 m by 1.8 m. A comprehensive list of treatments, including common names, product names, manufacturer information, and application rates, is provided in Table 2. All herbicide applications were made with a CO₂-pressurized hooded boom sprayer equipped with two TTI11003 flat fan nozzles (TeeJet®, Wheaton, IL), spaced 36 cm apart, calibrated to deliver 281 L ha⁻¹ of spray solution at 4.8 km hr⁻¹. Japanese stiltgrass was 4- to 6-tiller stage at the time of herbicide application. The experiment sites were mowed regularly at 1 wk intervals to a height of 6.4 cm throughout the study period.

Tall fescue and Japanese stiltgrass cover, control, and injury were visually assessed on a scale of 0 to 100%, with 0% being no control, no cover, or no injury and 100% being complete plant death or complete cover. Data were assessed at 0, 1, 2, 4, 6, and 8 wk after initial treatment (WAIT). Japanese stiltgrass counts were taken in each plot at 8 WAIT using a 1 m² quadrant. Visual cover of tall fescue or Japanese stiltgrass was converted to relative cover based on the percent cover of nontreated control within each replication. All response variables were subjected to analysis of variance (ANOVA) using PROC GLM in SAS v 9.3 (SAS Institute, Cary, NC). Treatment was considered as a fixed effect, while experimental run and block were treated as a random effect. The mean square of the treatment effect was tested for all assessed parameters using the mean square associated with the experimental run (McIntosh 1983). Means were separated using Fisher's protected LSD ($\alpha = 0.05$). Means were presented separately by experimental run if the treatment-by-experimental run interactions were significant, otherwise, means were pooled over experimental runs.

Assessing Fenoxaprop Combinations and Fluazifop for Japanese Stiltgrass Control in Kentucky Bluegrass. Four field experiments were conducted to assess Japanese stiltgrass control and Kentucky bluegrass tolerance after selective herbicide treatments in 2015 and 2017 (Table 1). The experimental design was a randomized complete block design with three replications and two temporal runs for each species. Treatments included a nontreated control; a premix of fenoxaprop + fluroxypyr + dicamba at 421 g ha⁻¹ (with or without nonionic surfactant); fluazifop at 53 g ha⁻¹ with nonionic surfactant; fluazifop at 105 g ha⁻¹ with nonionic surfactant; and quinclorac at 840 g ha⁻¹ with methylated seed oil. A detailed list of herbicide common names, trade names, manufacture details, and rates evaluated is provided in Table 3. Herbicide application method, site maintenance, and data collection were the same as the previous study except that digital images were also taken to quantify turf cover using TurfAnalyzer (Green Research Services, LLC, Fayetteville, AR) to detect the green pixels in each image. Turf cover was converted to relative cover based on the percent green cover in the nontreated plot within each replication. Data were analyzed similarly to the above-mentioned procedure as the experimental design and response variables are similar for both studies. Means were separated using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Initial Screen of POST Herbicides for Japanese Stiltgrass Control in Tall Fescue. The main effect of treatment was highly significant for visual injury on tall fescue ($P < 0.0001$) and not dependent on trial ($P > 0.05$), leading to the pooling of data across all four experimental runs (data not shown). Only treatments containing mesotrione caused injury to tall fescue, with injury levels between 15% and 25% at 2 WAIT. These treatments, however, did not affect the relative turf cover (data not shown). Transient injury from mesotrione on tall fescue has been noted in other studies (Goddard et al. 2010; Willis et al. 2006), while triclopyr showed no injury (Dernoeden et al. 2008). Fenoxaprop did not alter tall fescue color or density beyond commercially acceptable levels (McCarty et al. 1989) and was generally safe on turf even with frequent applications (Johnson and Carrow 1995). Similar to previous reports, treatments with topramezone and quinclorac resulted in less than 7% injury to tall fescue without impacting turf quality (Brewer et al. 2017; Patton et al. 2021).

The herbicide treatment-by-experimental run interaction was significant for Japanese stiltgrass control ($P < 0.0001$), relative weed cover ($P = 0.0032$), and weed shoot density ($P < 0.0001$) at 8 WAIT (Table 4). In nontreated control plots, Japanese stiltgrass density was 224 and 314 shoots m^{-2} at Newport and Blacksburg, respectively (Table 4). Fenoxaprop treatments, irrespective of rate, controlled Japanese stiltgrass $\geq 90\%$ and reduced relative cover to $< 15\%$ and ≤ 6 shoots m^{-2} at both study sites (Table 4). This aligns with previous research in forest ecosystems, which found that even reduced rates of fenoxaprop were as effective as the labeled rate for controlling Japanese stiltgrass (Peskin et al. 2005; Ward and Mervosh 2012). Topramezone, applied at 27 or 54 $g\ ha^{-1}$ (alone or with triclopyr), controlled Japanese stiltgrass $\geq 80\%$, reducing relative weed cover and shoot density $\leq 22\%$ and 35 shoots m^{-2} , respectively (Table 4). Although triclopyr did not increase topramezone performance for Japanese stiltgrass control (Table 4), both triclopyr and metribuzin have sustained or enhanced topramezone performance for goosegrass control (Brewer et al. 2022; Cox et al. 2017). Topramezone is specifically labeled for Japanese stiltgrass control in cool-season turf and recommended for selective management of troublesome weeds (Anonymous 2022b; Cox et al. 2017; Peppers et al. 2023; Landschoot et al. 2023). Treatments with mesotrione, quinclorac, and triclopyr decreased Japanese stiltgrass relative cover and shoot density but weed control was $\leq 75\%$ (Table 4). Other

studies have similarly reported poor control of Japanese stiltgrass following applications of quinclorac on golf course naturalized areas and triclopyr on cool-season grass forages (Flessner et al. 2019; Weaver et al. 2020). Overall, these data suggest that fenoxaprop and topramezone-based treatments effectively and selectively controlled Japanese stiltgrass in tall fescue lawns without causing significant turfgrass injury.

Assessing Fenoxaprop Combinations and Fluazifop for Japanese Stiltgrass Control in Kentucky Bluegrass. The main effect of treatment was significant for both Kentucky bluegrass injury ($P = 0.0004$) and relative turfgrass cover ($P = 0.039$) at 4 wk after treatment (WAT), but these response variables were not dependent ($P > 0.05$) on the experimental run (Table 5). Treatments containing fluazifop injured Kentucky bluegrass $\geq 25\%$ and reduced the digitally assessed relative turf cover WAT $\geq 20\%$ (Table 5). Fluazifop resulted in 25 to 46% Kentucky bluegrass injury, similar to previous reports (Warren et al. 1989).

In contrast, Kentucky bluegrass was highly tolerant to applications of dicamba + fenoxaprop + fluroxypyr (regardless of surfactant) or quinclorac; injury measured $\leq 3\%$ (Table 5). Quinclorac, when applied at 840 g ha^{-1} , did not cause injury to newly established Kentucky bluegrass (Reicher et al. 1999). Previous research also confirmed that tank mixing fenoxaprop with fluroxypyr does not compromise the efficacy of fenoxaprop on smooth crabgrass, nor does it cause injury to cool-season turfgrass (McCullough et al. 2009).

The main effect of treatment was significant for Japanese stiltgrass control ($P = 0.0002$), relative weed cover ($P = 0.0003$), and weed shoot density ($P < 0.0001$) at 8 WAT, with these response variables showing no dependence on the experimental run, therefore, data was pooled over runs (Table 5). At 8 WAT, the combination of dicamba + fenoxaprop + fluroxypyr controlled Japanese stiltgrass $\geq 92\%$, reduced relative cover and shoot density to $\leq 7\%$ cover and $< 5 \text{ shoots m}^{-2}$, respectively, both with and without nonionic surfactant (Table 5). In other work, fenoxaprop alone controlled Japanese stiltgrass 93% and reduced weed cover 89% (Judge et al. 2005a, 2005b). A commercial premix of dicamba, fenoxaprop, and fluroxypyr is marketed for controlling Japanese stiltgrass and other problematic weeds in Kentucky bluegrass turf (Anonymous 2017). Although the current study shows that dicamba and fluroxypyr do not reduce fenoxaprop performance for Japanese stiltgrass control, mixtures with other herbicides,

such as 2,4-D and mecoprop, have antagonized graminicides for annual grass control in other studies (Cox and Askew 2014).

Fluazifop treatments were also highly effective, controlling Japanese stiltgrass $\geq 85\%$ across different application rates, while reducing relative cover and weed shoot density to $\leq 17\%$ and < 20 shoots m^{-2} , respectively, at 8 WAT (Table 5). Similar results were observed by Judge et al. (2005b), who reported 97% control of Japanese stiltgrass 8 wk post-treatment with fluazifop. In the studies reported here, treatments were applied in July or August when Japanese stiltgrass was relatively mature and chances of subsequent germination were relatively low. Applications made earlier in the season may not perform as well due to subsequent seedling emergence as has been shown with Japanese stiltgrass (Judge et al. 2005b) and other grassy weeds (Askew et al. 2000). Quinclorac reduced Japanese stiltgrass relative cover by only 35% and shoot density to only 108 shoots m^{-2} , indicating it is not effective in controlling this weed (Table 5). Fluazifop at 53 g ha^{-1} injured Kentucky bluegrass 25% at 4 WAT, but low injury would not be considered completely safe by turfgrass managers. Additionally, this response is very different from the minimal injury observed on Kentucky bluegrass after dicamba + fenoxaprop + fluroxypyr treatments.

Practical Implications. This research provides information that will aid practitioners to selectively control Japanese stiltgrass in cool-season turfgrasses. Fenoxaprop selectively controls Japanese stiltgrass even at 35 g ha^{-1} and reduces the overall cost of managing this problematic weed in tall fescue. Topramezone-based programs also effectively control Japanese stiltgrass without compromising tall fescue safety. Fluazifop at 53 g ha^{-1} transiently injured Kentucky bluegrass but controlled Japanese stiltgrass effectively. Reduced rates of fluazifop could be adopted for selectively managing Japanese stiltgrass in Kentucky bluegrass if practitioners are willing to tolerate transient turf injury up to 4 wk after herbicide application. Dicamba, fenoxaprop, and fluroxypyr premix controlled Japanese stiltgrass $> 90\%$ without compromising Kentucky bluegrass safety. Although the tolerance of tall fescue to the dicamba, fenoxaprop, and fluroxypyr premix has not been evaluated in our study, this product is labeled for use on tall fescue and other cool-season turfgrass species.

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Competing Interests

The authors declare none.

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Table 1. List of field studies conducted in Virginia from 2014 to 2019 to evaluate postemergence (POST) herbicides for Japanese stiltgrass control in cool-season turfgrass lawns.

Study	Species assessed	Location	GPS coordinates	Herbicide application
POST herbicides for Japanese stiltgrass control in tall fescue ^a	Japanese stiltgrass and tall fescue	Newport, VA	37.29°N, 80.51°W	August 15, 2014
	Japanese stiltgrass and tall fescue	Blacksburg, VA	37.21°N, 80.41°W	July 15, 2015
	Tall fescue	Blacksburg, VA	37.22°N, 80.41°W	August 28, 2017
	Tall fescue	Blacksburg, VA	37.23°N, 80.44°W	August 15, 2019
Fenoxaprop combinations and fluazifop for Japanese stiltgrass control in Kentucky bluegrass	Japanese stiltgrass	Blacksburg, VA	37.23°N, 80.43°W	July 15, 2015
	Japanese stiltgrass	Newport, VA	37.29°N, 80.50°W	August 25, 2017
	Kentucky bluegrass	Blacksburg, VA	37.23°N, 80.43°W	July 15, 2015
	Kentucky bluegrass	Blacksburg, VA	37.22°N, 80.41°W	August 25, 2017

^aSelected herbicides were applied sequentially at a 3-week interval during the study

Table 2. List of herbicide common names, trade names, manufacturer's description, and rates used in field experiments to assess tall fescue tolerance and Japanese stiltgrass control.

Common name	Product name	Manufacturer	Rate
			g ae or ai ha ⁻¹
Fenoxaprop	Acclaim [®] Extra	Bayer Environmental Science, Cary, NC 27513	35
Fenoxaprop	Acclaim [®] Extra	Bayer Environmental Science, Cary, NC 27513	70
Fenoxaprop	Acclaim [®] Extra	Bayer Environmental Science, Cary, NC 27513	140
Mesotrione ^{a*}	Tenacity [®]	Syngenta Crop Protection, LLC, Greensboro, NC 27419	140
Mesotrione ^a	Tenacity [®]	Syngenta Crop Protection, LLC, Greensboro, NC 27419	280
Mesotrione triclopyr ^a	+ Tenacity [®] ; Turflon [®] Ester Ultra	Syngenta Crop Protection, LLC, Greensboro, NC 27419; Dow AgroSciences LLC, Indianapolis, IN 46268	280 + 1120
Quinclorac ^{b*}	Drive [®] XLR8	BASF Corp, Research Triangle Park, NC 27709	660
Quinclorac ^b	Drive [®] XLR8	BASF Corp., Research Triangle Park, NC 27709	1120
Topramezone ^{b*}	Pylex [™]	BASF Corp., Research Triangle Park, NC 27709	27
Topramezone ^b	Pylex [™]	BASF Corp., Research Triangle Park, NC 27709	54
Topramezone triclopyr ^b	+ Pylex [™] ; Turflon [®] Ester Ultra	BASF Corp., Research Triangle Park, NC 27709; Dow AgroSciences LLC, Indianapolis, IN 46268	54 + 1120
Triclopyr	Turflon [®] Ester Ultra	Dow AgroSciences LLC, Indianapolis, IN 46268	1120

^aNonionic surfactant at 0.25% v v⁻¹ was added

^bMethylated seed oil was added at 0.5% v/v

*Sequential applications were applied at a 3-week interval

Table 3. List of herbicide common names, trade names, manufacturer's description, and rates used in field experiments to assess the response of Kentucky bluegrass (*Poa pratensis*) and Japanese stiltgrass.

Common name	Product name	Manufacturer	Rate
			g ae or ai ha ⁻¹
Dicamba + fenoxaprop + fluroxypyr	Last Call™	Nufarm Americas Inc., Alsip, IL 60803	421
Dicamba + fenoxaprop + fluroxypyr ^a	Last Call™	Nufarm Americas Inc., Alsip, IL 60803	421
Fluazifop ^a	Ornamec®	PBI Gordan Corp., Kansas City, MO 64101	53
Fluazifop ^a	Ornamec®	PBI Gordan Corp., Kansas City, MO 64101	105
Quinclorac ^b	Drive® 75 DF	BASF Corp., Research Triangle Park, NC 27709	840

^aNonionic surfactant at 0.25% v/v was added

^bMethylated seed oil was added at 0.5% v/v

Table 4. Effect of herbicide treatments on Japanese stiltgrass control, relative cover, and shoot density at 8 weeks after initial treatment.^{ab*}

Treatment	Rate g ae or ai ha ⁻¹	Japanese stiltgrass control				Japanese stiltgrass relative cover				Japanese stiltgrass shoot density			
		Newport		Blacksburg		Newport		Blacksburg		Newport		Blacksburg	
		%				%				no m ⁻²			
Nontreated		-	-	-	-	-	-	-	-	224	a	314	a
Fenoxapro p	35	90	bc	97	a	14	cd	5	c	6	f	5	c
Fenoxapro p	70	95	ab	98	a	7	de	3	c	2	f	2	c
Fenoxapro p	140	10	a	99	a	0	de	1	c	0	f	1	c
Mesotrione a*	140	31	h	13	e	53	a	82	a	130	b	293	a
Mesotrione a	280	40	g	24	e	51	a	74	a	122	bc	265	a
Mesotrione + triclopyr	280 + 1120	75	d	62	b	32	abc	46	b	81	d	162	b
Quinclorac b*	660	62	e	55	bc	32	abc	46	b	87	cd	160	b
Quinclorac b	1120	50	f	35	d	42	ab	80	a	105	bcd	250	a
Topramezo ne ^{b*}	27	87	cd	94	a	22	bcd	4	c	29	f	7	c
Topramezo ne ^b	54	80	d	91	a	21	bcd	11	c	34	ef	10	c
Topramezo ne + triclopyr ^b	54 + 1120	87	cd	97	a	21	bcd	3	c	26	f	3	c
Triclopyr	1120	67	e	43	cd	36	ab	63	ab	69	de	173	b
<i>P</i> -value		<0.0001				0.0032				<0.0001			

Note: Means followed by a different letter within the same column are different based on Fisher's protected LSD ($\alpha = 0.05$)

^aNonionic surfactant at 0.25% v v⁻¹ was added

^bMethylated seed oil was added at 0.5% v/v

*Sequential applications were applied at a 3-week interval

Table 5. Effect of herbicide treatments on Kentucky bluegrass injury and relative cover compared to nontreated control at 4 weeks after treatment (WAT) and Japanese stiltgrass (*Microstegium vimineum*) control, relative weed cover, and shoot density at 8 WAT.^{abc*}

Treatment	Turf injury WAT		Relative turf cover 4 WAT		Japanese stiltgrass control WAT		Japanese stiltgrass 8 relative cover 8 WAT		Japanese stiltgrass shoot density 8 WAT	
	%		%		%		%		no m ⁻²	
Nontreated	-	-	-	-	-	-	-	-	228	a
Dicamba + fenoxaprop fluroxypyr	+ 2	c	105	a	97	a	2	c	2	c
Dicamba + fenoxaprop fluroxypyr ^a	+ 1	c	101	a	92	ab	7	c	5	c
Fluazifop ^{ab}	25	b	80	b	85	b	17	b	19	c
Fluazifop ^{ac}	46	a	75	b	87	b	9	bc	7	c
Quinclorac ^d	3	c	104	a	42	c	65	a	108	b
<i>P</i> -value	0.0004		0.039		0.0002		0.0003		<0.0001	

^aNonionic surfactant at 0.25% v v⁻¹ was added

^bFluazifop was applied at 53 g ha⁻¹

^cFluazifop was applied at 105 g ha⁻¹

^dMethylated seed oil was added at 0.5% v/v

*Means followed by the same letter within each column are not different based on Fisher's protected LSD ($\alpha = 0.05$)