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Dithiopyr Controls Common Lespedeza (*Kummerowia striata*) in Bermudagrass

Diego Gómez de Barreda, Rashmi Singh, Sudeep S. Sidhu, and Patrick E. McCullough*

Common lespedeza is a problematic summer annual weed in bermudagrass lawns, with limited PRE herbicides available for control. Dithiopyr is a pyridine herbicide primarily used for PRE grassy weed control but has shown potential efficacy for controlling annual legumes. The objectives of this research were to evaluate efficacy and behavior of dithiopyr in common lespedeza. In a 3-yr field experiment, sequential dithiopyr applications at 0.42 or 0.56 kg ai ha^{-1} beginning in late winter and single applications of dithiopyr at 0.56 kg ai ha^{-1} in spring controlled common lespedeza ≥ 88%. Single and sequential applications of indaziflam at 0.035 and 0.053 kg ai ha^{-1} provided poor control (< 70%) of common lespedeza by late summer. In laboratory experiments, bermudagrass and common lespedeza had similar foliar absorption of ^{14}C-dithiopyr, averaging 10% of the ^{14}C applied, and both species retained > 80% of ^{14}C in the treated leaf at 72 h after treatment (HAT). Common lespedeza translocated 6 times more root-absorbed ^{14}C to shoots than bermudagrass and had 2.8 times greater absorption (Bq mg^{-1}) at 72 HAT. In metabolism experiments, parent herbicide levels measured ≥ 84% of extracted ^{14}C in both species at 1, 3, and 7 d after treatment. Overall, dithiopyr effectively controls common lespedeza in bermudagrass as a PRE treatment in spring. Susceptibility of common lespedeza to dithiopyr is associated with acropetal translocation and greater herbicide concentrations compared with a tolerant species, bermudagrass.

Nomenclature: Common lespedeza (*Kummerowia striata* (Thunb.) Schindl.) 'Kobe'; bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burtt-Davy) 'Princess 77'.

Key words: Absorption, efficacy, metabolism, translocation, turfgrass.

In Georgia, common lespedeza is a problematic summer annual weed in turfgrass. Common lespedeza is a freely branched legume that has competitive growth with turfgrasses during spring and summer. As plants mature, common lespedeza produces woody stems that enhance tolerances to drought, heat, and other stresses (Hein and Vinall 1933; Nakata 1952). In Georgia, common lespedeza emerges from seed in spring during bermudagrass transition from winter dormancy. Preventing the establishment of common lespedeza with PRE herbicides is desirable to enhance bermudagrass competition with summer weeds and reduce POST herbicide use in management programs.

PRE applications of atrazine may effectively control common lespedeza in warm-season turfgrasses (Johnson 1979). However, atrazine provides short-term PRE control (4 to 6 wk) and sequential applications needed for common lespedeza control may excessively injure bermudagrass (Johnson 1975, 1979). Isoxaben is a cellulose biosynthesis inhibitor that provides PRE control of annual broadleaf weeds such as henbit (*Lamium amplexicaule* L.), corn speedwell (*Veronica officinalis* L.), and spotted spurge (*Euphorbia maculata* (L.) Small) (Chandran et al. 1998; Sabba and Vaughn 1999). However, economics, inconsistent efficacy, and limited control of grassy weeds are major limitations for isoxaben use in turfgrass (Derr 2002; Neal and Senesac 1990).

Dinitroanilines (DNAs) are PRE herbicides that are widely used for controlling summer annual grassy weeds, such as smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl) and goosegrass (*Eleusine indica* (L.) Gaertn.), in turfgrass (Dernoeden et al. 1993; Gasper et al. 1994). DNAs inhibit tubulin polymerization during mitosis, resulting in disfigured, club-shaped roots that prevent establishment of susceptible species (Ambruster et al. 1990; Hoffman and Vaughn 1994; Vaughn and Lehnen 1991). However, DNA herbicides have inconsistent efficacy for PRE control of annual legumes and POST herbicides are often needed for control (Wyse and McGraw 1987).

Dithiopyr is a pyridine herbicide that provides PRE control of crabgrass (*Digitaria* spp.) and other grassy weeds in turf (Enache and Ilnicki 1991; Johnson and Murphy 1993; Johnson 1997a, b). Dithiopyr inhibits mitosis in susceptible species, and is primarily used for PRE grassy weed control in

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*Associate professor, Universitat Politècnica de València, Camino de Vera s/n, Edificio 3P, 46022 Valencia, Spain; former Postdoctoral Researcher, former Postdoctoral Researcher, and Associate Professor, University of Georgia, Griffin, GA 30223. Corresponding author's E-mail: pmccull@uga.edu.

the U.S. transition zone and cool-humid region (Ambruster et al. 1990; Hoffman and Vaughn 1994; Vaughn and Lehnen 1991). Dithiopyr is absorbed by roots and shoots and has significant acropetal translocation in susceptible grassy weeds (Pyon et al. 1994). However, the physiological behavior in legumes has received limited investigation.

Although dithiopyr is primarily used for grassy weed control, PRE applications have shown to effectively control common purslane (*Portulaca oleracea* L.), spotted spurge, yellow woodsorrel (*Oxalis stricta* L.), and other broadleaf species (Baldos et al. 2010; Derr 1994). In preliminary field experiments, sequential dithiopyr treatments at 0.42 kg ai ha^{-1} in spring provided excellent control of common lespedeza (> 90%) in bermudagrass after 6 mo and were more effective than a DNA herbicide, prodiamine (unpublished data).

Dithiopyr may have potential to control common lespedeza in bermudagrass lawns as an alternative to other PRE herbicides. However, further investigation is needed to determine rates and regimens that effectively control common lespedeza in turfgrass. Moreover, differential behavior of dithiopyr in grasses and legumes has received limited investigation. The objectives of this research were to (1) evaluate rates and timings of dithiopyr applications in spring for PRE control of common lespedeza in bermudagrass, and (2) evaluate physiological behavior of dithiopyr in bermudagrass and common lespedeza.

Materials and Methods

Field Experiments. Experiments were conducted in Griffin, GA (33°15′N, 84°16′W) from February to September 2011, 2012, and 2013. Turf was common bermudagrass (unknown cultivar) grown on a Cecil sandy loam with a 6.0 pH and 2% organic matter. Plots used in each year were adjacent to plots in previous experiments. Bermudagrass was mowed weekly during active growth at a 5-cm height with a rotary mower, and clippings returned. Bermudagrass was irrigated as needed to prevent wilting.

Experimental design was a randomized complete block with four replications of 1.5- by 3-m plots. Six treatments were evaluated and included sequential applications of dithiopyr at 0.42 or 0.56 kg ai ha^{-1}, single applications of dithiopyr at 0.56 kg ha^{-1}, sequential indaziflam applications at 0.035 or 0.053 kg ai ha^{-1}, or single indaziflam applications at 0.053 ka ha^{-1}. Sequential dithiopyr and indaziflam treatments were initially applied at a PRE timing for summer annual weed control in Griffin, GA. Sequential applications were made 8 and 12 wk after initial treatments for dithiopyr and indaziflam, respectively, as recommended on product labels for PRE annual weed control (Anonymous 2006, 2014). Single treatments were applied later in spring to simulate delayed PRE applications for summer annual weed control. Application dates are presented in Table 1. Treatments were applied with CO_2-pressured sprayers calibrated to deliver 234 L ha^{-1} with three 8004 nozzles (flat-fan, TeeJet Spraying Systems Co., Roswell, GA 30075).

Common lespedeza cover in nontreated plots was visually evaluated on a percent scale where 0 = no cover and 100 = complete plot cover in June, July, and August/September on dates in Table 1. Common lespedeza control in treated plots was visually evaluated on these dates using a percent scale where 0 equaled no reductions in cover from the nontreated and 100 equaled complete reductions from the nontreated by replication. Turfgrass injury was measured visually on a percent scale where 0 equaled no injury relative to the nontreated, and 100 equaled completely necrotic. Data are presented by evaluation month due to inconsistencies in rating dates over the 3 yr.

Foliar Absorption and Translocation. Common lespedeza (Adams-Briscoe, Jackson, GA 30233) and 'Princess-77' bermudagrass (Pennington Seed, Inc., Madison, GA 30650) were seeded in separate pots measuring 20 cm depth by 3.8 cm in diameter with sand:peat (80 : 20 v/v) in a greenhouse set for 32/25 C (day/night). Plants were irrigated to promote establishment and bermudagrass was trimmed at a 5-cm height with sheers as needed. Plants selected for treatments included bermudagrass at the three- to five-tiller stage, and common lespedeza at seedling stage with one to two fully expanded leaves. The tillered stage of bermudagrass was chosen to mimic established turfgrass that would be suitable for dithiopyr treatment.

Plants were placed in a growth chamber (Percival Scientific, 505 Research Drive, Perry, IA 50220) set for 25/20 C (day/night) with approximately 50% relative humidity and 12-h photoperiods of 350 μmol m^{-1} s^{-1}. Plants were irrigated to prevent wilt and acclimated in the growth chamber for 5 d before treatments. A broadcast application of dithiopyr was made at 0.56 kg ai ha^{-1} at 374 L ha^{-1} with the aforementioned CO_2-pressured sprayers. Soil was left uncovered for

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Table 1. Common lespedeza control after herbicide treatments in three field experiments, 2011–2013, Griffin, GA. Results were pooled over the three experiments.

Initial treatment[a]	Herbicide	Rate	Common lespedeza control		
			June[b]	July	August/September
		kg ai ha^{-1}		%	
Late winter	Dithiopyr	0.42 fb 0.42	100	100	100
		0.56 fb 0.56	100	100	98
	Indaziflam	0.035 fb 0.035	86	73	68
		0.053 fb 0.053	71	63	51
Spring	Dithiopyr	0.56	97	95	88
	Indaziflam	0.053	49	29	30
		$LSD_{0.05}$	19	19	27

[a] Application dates in 2011 for late-winter dithiopyr treatments were February 22 followed by (fb) April 19; February 22 fb May 16 for indaziflam treatments; and spring treatments were applied April 19 for both herbicides. Application dates in 2012 for late-winter dithiopyr treatments were March 13 fb May 8; March 13 fb May 16 for indaziflam treatments; and spring treatments were applied April 9 for both herbicides. Application dates in 2013 for late-winter dithiopyr treatments were February 25 fb April 24; February 25 fb May 21 for indaziflam treatments; and spring treatments were applied April 24 for both herbicides.

[b] Rating dates were June 21, 2011, June 6, 2012, and June 17, 2013; July 18, 2011, July 5, 2012, and July 15, 2013; and August 23, 2011, September 24, 2012, and September 9, 2013. Common lespedeza cover in nontreated plots averaged 12 (± 2), 16 (± 3), and 21% (± 6%) on evaluations in June, July, and August/September, respectively (Table 1).

treatments. Immediately after broadcast applications, two 1-µl droplets of ^{14}C-dithiopyr (specific activity: 27 mCi/mmol, 4-^{14}C pyridine ring labeled, 96% radiochemical purity) were applied to the first fully expanded leaf containing 333 Bq of radioactivity. Appropriate amount of formulated dithiopyr (Dimension 2EW, Dow AgroSciences LLC, Indianapolis, IN 46268) was added to the spotting solution to simulate 0.56 kg ha^{-1} at 374 L ha^{-1}. A nonionic surfactant (Activator 90, Loveland Products, Inc., Greeley, CO 80632-1286) was included in the spotting solution at 0.125% to facilitate deposition of the droplet.

Plants (roots + shoots) were harvested at 1, 4, 8, 24, or 72 h after treatment (HAT). Treated leaves were excised from the base of the plant and rinsed with 5 ml of 20% methanol solution in a 20-ml scintillation vial. For the 72-h harvest, plants were separated into treated leaves, nontreated shoots, and roots. Samples were oven dried at 50 C for 72 h and then combusted in a biological oxidizer (Model OX-500, R. J. Harvey Instrument Corp., Hillsdale, NJ 076742). Radioactivity was then quantified using liquid scintillation counting (Model LS6500, Beckman-Coulter, Inc., Fullerton, CA 92834-3100).

Root Absorption and Translocation. Pots were seeded with the aforementioned soil, methods, and greenhouses. Single tillers of bermudagrass were chosen for treatments and common lespedeza was at the aforementioned seedling stage. Plant roots were washed free of soil, and individual plants were grown hydroponically in 0.9 L of half-strength Hoagland's solution in a 1-L plastic tank. The tank was covered with aluminum foil, and plants were placed through holes to facilitate root submergence in solution. An aquarium pump was used to provide oxygen to the solution. The tank was then placed in the aforementioned growth chamber for 72 h to acclimate plants to chamber conditions and hydroponic culture.

Plants were then removed from the solution and placed individually in 50-ml plastic tubes containing 25 ml of half-strength Hoagland's solution supplemented with 20 Bq ml^{-1} of ^{14}C-dithiopyr. Formulated dithiopyr (2EW) was added to simulate a 0.56 kg ha^{-1} surface application rate. Tubes were covered with aluminum foil and cotton balls were placed at the base of plants to facilitate root submergence in solution.

Plants were harvested at 72 HAT and roots were rinsed with 20% methanol solution to remove unabsorbed ^{14}C. Roots were blotted with a paper towel, and separated from shoots with sheers. Samples were then oven dried at 50 C for 72 h, weighed, combusted in a biological oxidizer, and radioactivity was quantified with a liquid scintillation counter.

Metabolism. Common lespedeza and Princess-77 bermudagrass were seeded in containers with the aforementioned materials and methods. Plants were placed in a growth chamber set for 25/20 C (day/night) with approximately 50% relative humidity and 12-h photoperiods of 350 µmol m^{-1} s^{-1}.

Plants were irrigated to prevent wilt and allowed to resume active growth in the growth chamber for 5 d before treatments. A broadcast application of dithiopyr was made at 0.56 kg ai ha^{-1} at 374 L ha^{-1} with the aforementioned CO_2-pressured sprayers. Immediately after broadcast applications, two 1-µl droplets of ^{14}C-dithiopyr were applied containing 2 kBq of radioactivity. Appropriate amount of formulated dithiopyr was added to the spotting solution to simulate 0.56 kg ha^{-1} at 374 L ha^{-1}. A nonionic surfactant (Activator 90, Loveland Products) was included in the spotting solution at 0.125% to facilitate deposition of the droplet.

Treated leaves were excised at 1, 3, or 7 d after treatment (DAT). Unabsorbed ^{14}C-dithiopyr was removed by swirling the treated leaf in a 20-ml scintillation vial containing 2 mL of a 10% methanol solution for approximately 45 s. Upon removal from vials, leaves were rinsed with approximately 2 ml of 20% methanol solution and stored at −20 C until further analysis.

The treated leaf was chosen for metabolism evaluations since < 20% of absorbed radioactivity was translocated in absorption experiments. For extraction, leaves were placed in 1.5-ml tubes, ground with liquid nitrogen, and then filled with 1 ml of methanol. Tubes were then agitated on a rotary shaker for 30 s and placed in water sonication for 24 h. Samples were then centrifuged for 5 m at 13,500 × *g* and solution was transferred to separate tubes. In pilot experiments, leaf residue was oxidized and ^{14}C extraction measured > 90% for all species based on total radioactivity recovered (data not shown).

A 450-µl aliquot of extraction solvent was spotted on 20-cm by 20-cm silica gel plates and developed in glass chambers with 1 : 8 ethyl acetate : hexane. Peaks in radioactivity were identified with a radiochromatogram scanner (BioScan AR-2000, Bioscan, 4590 MacArthur Boulevard NW, Washington, DC 20007) equipped with Laura Chromatography Data Collection and Analysis Software® (LabLogic Systems, Inc. 1040 E. Brandon Blvd., Brandon, FL 33511-5509). To identify the parent peak, stock ^{14}C-dithiopyr was added to methanol solution with ground leaves and run through extraction procedures. A 250-µl aliquot from these samples was developed on thin-layer chromatography plates and location of the parent herbicide was similar to ^{14}C-dithiopyr without ground tissue.

Experimental Design and Data Analysis. The experimental design of the field experiment was a randomized complete block with four replications and the experiment was repeated twice. The experimental design of all laboratory experiments was completely randomized with four replications and experiments were repeated.

In foliar uptake experiments, percent absorption was calculated by dividing the total radioactivity recovered in samples by the initial amount applied to the leaf. Percent translocation was determined by dividing the radioactivity recovered in samples by the total recovered in whole plants. In root uptake experiments, specific radioactivity in roots and shoots was calculated by dividing radioactivity per sample by dry mass. Percent distribution was determined by dividing the radioactivity recovered in samples by the total amount recovered in the plant. Metabolism data are presented as the percentage of extracted ^{14}C as the parent herbicide.

Data were subjected to ANOVA with the general linear model procedure in SAS (SAS Institute v. 9.2, Cary NC 27513). Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$. Experiment-by-treatment and experiment-by-species interactions were not detected, and thus, results were pooled over experiment repetitions.

Results and Discussion

Field Experiments. No treatment injured bermudagrass (data not shown). Common lespedeza was not present in the field until May of all years, suggesting that germination did not occur in March or April. Cover in nontreated plots averaged 12 (± 2), 16 (± 3), and 21% (± 6) on evaluations in June, July, and August/September, respectively (Table 1). Sequential dithiopyr treatments at both rates provided > 97% control of common lespedeza on all observation dates. Sequential applications of indaziflam averaged 77% control of common lespedeza in June, but control declined from both treatments to < 70% by the last observation in late summer. Single applications of dithiopyr in spring controlled common lespedeza > 87% on all evaluation dates. Single indaziflam applications at 0.053 kg ha^{-1} provided < 50% control of common lespedeza on all dates when applied at the spring timing.

Efficacy of PRE herbicides for controlling common lespedeza has received limited investigation in turfgrass. Practitioners in the southern United States typically apply PRE herbicides in late winter to control crabgrass, but most PRE herbicides are ineffective for controlling legumes.

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Table 2. Absorption, distribution, and specific radioactivity after root applications of ^{14}C-dithiopyr to 'Princess-77' bermudagrass and common lespedeza in two combined experiments, 2012, Griffin, GA.

Species	^{14}C distribution		Specific radioactivity		
	Roots	Shoots	Roots	Shoots	Total[a]
	% of absorbed		Bq mg^{-1}		
Bermudagrass	90	10	2.3	0.1	0.6
Common lespedeza	36	64	9.0	1.3	1.7
$LSD_{0.05}$	10	10	1.6	0.7	0.8

[a] Total radioactivity was calculated by dividing the sum radioactivity in roots and shoots by the sum weight of roots and shoots.

Common lespedeza often emerges in lawns or other turfgrass areas, and POST herbicides are needed for control (Boyd 2009; Johnson 1979; Wehtje 2008). Single applications of dithiopyr at 0.56 kg ha^{-1} in spring were equally effective for controlling common lespedeza as sequential treatments into late summer. Sequential indaziflam treatments appear to have potential to suppress or provide partial common lespedeza control in early summer, but these treatments provided poor control ($< 70\%$) by late summer in all years.

Turfgrass managers in Georgia often apply PRE herbicides to bermudagrass during spring green-up. Although atrazine may be an effective option for common lespedeza control, sequential applications required to extend residual control may be injurious to bermudagrass in spring (Johnson 1975, 1979). Dithiopyr did not injure bermudagrass over the 3-yr period and may be an effective option during transitional growth from winter dormancy. In other crops, researchers have noted that PRE applications of flumioxazin, metolachlor, and sulfentrazone controlled sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don) $> 86\%$ after 16 wk (Farris and Murray 2009). Perhaps these herbicides alone or in mixtures with other herbicides have potential for controlling common lespedeza and warrant further investigation in turfgrass.

^{14}C-Dithiopyr Absorption and Translocation. Bermudagrass and common lespedeza absorbed 4 to 10% of foliar-applied ^{14}C-dithiopyr from 1 to 72 HAT, but differences between species were not detected (data not shown). The majority ($\sim$90% of the maximum) of absorption was reached at approximately 24 HAT in both species. Bermudagrass and common lespedeza retained $\geq 82\%$ of absorbed ^{14}C in the treated leaf, and differences were not detected between species for translocation to nontreated shoots and roots (data not shown). Similar levels of foliar absorption and translocation have been reported with the DNA herbicides benefin, ethafluralin, and trifluralin (Jacques and Harvey 1979; Willis and Putnam 1986). Researchers have noted that these herbicides are absorbed by shoots but have minimal translocation and basipetal movement.

Dithiopyr had acropetal movement after root absorption in both species. However, common lespedeza translocated 6 times more ^{14}C to shoots than bermudagrass, measuring 64 and 10% respectively (Table 2). Common lespedeza recovered a total 1.7 Bq mg^{-1} and was 2.8 times greater than bermudagrass. Similar differences were detected in roots but common lespedeza had 13 times greater specific radioactivity in shoots than bermudagrass.

Common lespedeza roots appear to have greater affinity for dithiopyr absorption than bermudagrass and may be critical for control. Root uptake of DNA herbicide has been previously attributed to differential tolerance levels of various species. For example, Durgesha (1994) noted that slender amaranth (*Amaranthus viridis* L.) absorbed half as much ^{14}C-fluchloralin as a more susceptible species, peanut (*Arachis hypogaea* L.). Upadhyaya and Nooden (1987) reported that pea (*Pisum sativum* L.) root growth was less susceptible to inhibition by oryzalin than corn (*Zea mays* L.) because of less accumulation and a lower binding affinity.

Differential acropetal translocation levels have been previously attributed to dithiopyr selectivity for weed control. Pyon et al. (1994) noted that barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.] distributed more root-applied ^{14}C-dithiopyr to shoots than rice (*Oryza sativa* L.) at 72 HAT. The researchers surmised that greater translocation to shoots contributes to efficacy for selective barnyardgrass control. In smooth crabgrass, multileaf and multitiller plants distributed 43 and 20% of total absorbed ^{14}C to shoots from root applications of ^{14}C-dithiopyr, respectively (McCullough et al. 2014). These differences in translocation are associated with efficacy of dithiopyr for controlling multileaf smooth crabgrass, compared with tillered

Table 3. Metabolism of foliar-applied ^{14}C-dithiopyr in 'Princess-77' bermudagrass and common lespedeza in two experiments, 2012–2013, Griffin, GA. Results were pooled over experimental runs.

Species	Parent herbicide recovery		
	1 DAT[a]	3 DAT	7 DAT
	% of ^{14}C extracted		
Bermudagrass	93	90	84
Common lespedeza	97	95	95
$LSD_{0.05}$	4	2	4

[a] Abbreviation: DAT, days after treatment.

plants. Perhaps retention of dithiopyr in roots contributes to tolerance of turfgrasses and other species to applications. However, acropetal movement of dithiopyr to shoots of common lespedeza is comparable with previous reports in barnyardgrass and smooth crabgrass after root uptake.

Metabolism. The retention factor (R_f) for dithiopyr was detected at 0.55 and one additional peak was detected at R_f 0.1 in both species. At 1 DAT, parent dithiopyr levels in bermudagrass and common lespedeza measured 93 and 97% of total ^{14}C recovered, respectively (Table 3). Parent herbicide levels declined to 90 and 84% of extracted radioactivity in bermudagrass at 3 and 7 DAT, respectively, but common lespedeza had 95% parent herbicide recovered at both harvests. Although there was statistical separation between species, differences in parent herbicide recovery were probably not meaningful in terms of species tolerance levels to applications.

Selectivity of mitotic inhibitors has been attributed to herbicide binding in susceptible species rather than differential metabolism. Upadhyaya and Nooden (1980, 1987) found no metabolism of ^{14}C-oryzalin in corn and pea. Probst et al. (1967) noted that carrot (*Daucus carota* L.) produced one major metabolite of ^{14}C-trifluralin but the majority of radioactivity extracted was unaltered parent herbicide. Altered binding sites have conferred resistance to mitotic inhibitors in several species. Anthony et al. (1998) found that the molecular basis of DNA herbicide resistance in goosegrass [*Eleusine indica* (L.) Gaertn.] was due to a point mutation in α-tubulin, a microtubule cytoskeletal protein. Similarly, Hashim et al. (2011) found that resistance to trifluralin in shortawn foxtail (*Alopecurus aequalis* Sobol.) resulted from a similar mutation in α-tubulin. Further investigation is needed to evaluate differential binding of dithiopyr in turfgrasses, legumes, and other weedy species.

Overall, dithiopyr is an effective PRE herbicide for common lespedeza control in bermudagrass. Single and sequential dithiopyr applications at PRE annual grassy weed-control timings effectively controlled common lespedeza in bermudagrass. Differences in metabolism probably do not account for selectivity of dithiopyr for common lespedeza control in bermudagrass. However, greater herbicide uptake in seedling common lespedeza and distribution from roots to shoots appear to be associated with efficacy of spring PRE applications in bermudagrass. Further research is needed to evaluate efficacy of POST dithiopyr applications for common lespedeza control in turfgrass and efficacy for controlling other legumes.

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