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Soil Organic Matter Content and Volumetric Water Content Affect Indaziflam–Soil Bioavailability

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Indaziflam is a cellulose biosynthesis-inhibiting herbicide for annual weed control in various agricultural systems. Sporadic cases of unacceptable injury to desirable plants have been reported after indaziflam application, which may have been due to conditions favoring increased indaziflam–soil bioavailability. Research was conducted from 2013 to 2015 on a sandy soil to elucidate the effects of soil organic matter content (SOMC) and soil volumetric water content (SVWC) on indaziflam–soil bioavailability. Indaziflam was applied (50 or 100 g ha^{-1}) at fall only, fall plus spring, and spring only timings to plots in a factorial arrangement of SOMC, pre–indaziflam application (PrIA) SVWC, and post–indaziflam application (PoIA) SVWC. After application, field soil cores were collected for a subsequent greenhouse bioassay experiment, where foliage mass reduction of perennial ryegrass seeded from 0 to 15 cm soil depth was used as an indicator of indaziflam–soil bioavailability throughout the profile. Significant edaphic effects were observed at 0 to 2.5, 2.5 to 5, and 5 to 7.5 cm depths, with increased bioavailability at low compared with high SOMC. Pre–indaziflam application SVWC did not affect bioavailability, whereas PoIA high SVWC increased indaziflam–soil bioavailability at 2.5 to 7.5 cm depth compared with PoIA low SVWC. Low SOMC–PoIA high SVWC decreased perennial ryegrass foliage mass 40 and 37% at 5 to 7.5 cm depth from cores collected 10 and 14 wk after treatment, respectively, whereas reductions from all other SOMC–PoIA SVWC combinations were < 12% and did not vary from each other. Pearson's correlation coefficients showed a moderate, positive relationship between perennial ryegrass mass reductions at 0 to 2.5, 2.5 to 5, 0 to 5, and 0 to 10 cm depths and hybrid bermudagrass cover reduction, which suggests conditions favoring increased indaziflam–soil bioavailability can adversely affect plant growth. Data from this research will aid land managers to use indaziflam effectively without adversely affecting growth of desirable species.

Nomenclature: Indaziflam; hybrid bermudagrass, *Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt–Davey; perennial ryegrass, *Lolium perenne* L.

Key words: Application timing, bioassay, cellulose biosynthesis inhibition, herbicide injury, herbicide leaching.

Indaziflam (Weed Science Society of America [WSSA] group 29) is a selective PRE alkylazine herbicide that is currently registered for use in citrus fruits, commercial and residential areas, commercial nurseries, forestry sites, grapes, tree nuts, and turfgrass (Anonymous 2014, 2015; EPA 2010). Indaziflam inhibits cellulose biosynthesis in susceptible dicot and monocot species and provides an alternative mode of action for controlling dinitroaniline–resistant weeds (EPA 2010; McCullough et al. 2013). After U.S. registration in 2010, in situ reports surfaced alleging adverse effects on hybrid bermudagrass and pecan trees [*Carya illinoinensis* (Wangenh.) K. Koch] (González–Delgado et al. 2015; HGCSA 2011). Indaziflam injury has been described as curling of new growth, reddening of leaf tissue, and eventual necrosis on hybrid bermudagrass, and leaf necrosis and varying trunk injuries on pecan trees (González–Delgado et al. 2015; Jones et al. 2015).

Indaziflam is absorbed by plant roots, and conditions favoring increased soil bioavailability may attribute to cases where plant injury has occurred (Anonymous 2015; Brosnan and Breeden 2012; González-Delgado et al. 2015; Jeffries et al. 2016). Physicochemical properties pertaining to indaziflam–soil movement/bioavailability include the dissociation constant ($pK_a = 3.5$, weak acid), field half-life ($T_{1/2} > 150$ d), water solubility ($K_s =$ 2.4 to 4.4 mg L^{-1}; pH 5 to 9), and soil organic carbon–water partition coefficient ($K_{oc} = 434$ to 1,544 ml g^{-1}), which collectively suggest indaziflam may be mobile in soil because of long field persistence coupled with low aqueous solubility and moderate organic carbon sorption (Alonso et al. 2011; EPA 2010). This idea was supported by Jhala

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et al. (2012), who reported indaziflam (145 g ai ha^{-1}) leached 30 cm under 15 cm of simulated rainfall compared with 13 cm under 5 cm of simulated rainfall shortly after application, which was quantified with a soil bioassay via perennial ryegrass mortality.

Compared with analytical methods, bioassays are a less robust method for quantitative residue determination; however, they can effectively provide qualitative data comparing herbicide leaching and resistance with reduced resource inputs (Beckie et al. 2000; Jhala et al. 2012). Additionally, bioassay experiments are used to quantify relative herbicide–soil bioavailability. Gannon et al. (2014) reported saflufenacil–soil bioavailability (WSSA Group 14) measured 14 d after treatment via canola (*Brassica napus* L.) total plant fresh mass was positively correlated with soil organic matter ($r = 0.85$), humic matter ($r = 0.81$), and cation exchange capacity ($r = 0.49$).

In addition to moisture inputs affecting indaziflam–soil movement, edaphic conditions also influence indaziflam behavior. Specifically, plant injury may increase with increasing soil coarseness, decreasing soil organic matter content (SOMC), and increasing soil volumetric water content (SVWC) (González-Delgado et al. 2015; Jeffries and Gannon 2016; Jeffries et al. 2016; Jones et al. 2013a,b; Schneider et al. 2015). Minirhizotron research conducted by Jones et al. (2013b) determined indaziflam (35 to 70 g ha^{-1}) caused more hybrid bermudagrass injury 6 wk after treatment (WAT) when applied to a sand (99, 1, and 0% sand, silt, and clay, respectively) compared with a silt loam (28, 48, and 24% sand, silt, and clay, respectively). Schneider et al. (2015) reported similar findings with regard to soil texture, as well as improvements in hybrid bermudagrass color 5 wk after indaziflam application (16 and 48 g ha^{-1}) to sand and loamy sand with 2% (wt/wt) SOMC compared with each soil with 0% (wt/wt) SOMC. Results from field research comparing indaziflam application (65 g ha^{-1}) to hybrid bermudagrass in differing conditions also suggest that SOMC influences plant growth, because hybrid bermudagrass cover 12 WAT was threefold less in an area with 1.2% SOMC (wt/wt) (28% cover) compared with 2.5% SOMC (wt/wt) (92% cover); however, the authors also attributed these differing growth responses to reduced solar radiation in the lower SOMC system (Jeffries et al. 2016). González-Delgado et al. (2015) hypothesized indaziflam pecan tree injury was influenced by SOMC, with decreased SOMC allowing for increased plant uptake; however, the authors noted additional studies were required for confirmation. Jeffries and Gannon (2016) reported that hybrid bermudagrass cover reduction after indaziflam application (100 g ha^{-1}) was three- to sixfold greater 8 wk after spring treatment (WAST) in low compared with high SOMC (low and high SOMC were 1.4 and 5.5% (wt/wt), respectively, at 0 to 2.5 cm depth). Additionally, increasing SVWC after application doubled cover reduction in low SOMC, whereas high SOMC buffered the effect of soil moisture (Jeffries and Gannon 2016).

Previous research has shown that decreasing SOMC and increasing SVWC may increase herbicide bioavailability. Song et al. (2010) reported that organic matter amendments derived from sludge and straw decreased chlorotoluron soil bioavailability, which increased wheat (*Triticum aestivum* L.) tolerance by buffering herbicide-induced oxidative stress. Sun et al. (2012) conducted an experiment evaluating fluridone and norflurazon sorption in soil sediment, as well as organic matter fractions isolated from sediment. The authors reported that K_{oc} values across herbicides were an average of 15 and 30 times greater for isolated black carbon and humic acid, respectively, than for bulk sediment, indicating the importance of SOMC on herbicide–soil sorption (Sun et al. 2012). Pätzold and Brümmer (2003) reported extractable diuron, metolachlor, pendimethalin, and terbuthylazine increased as SVWC increased from 10 to 60% (v/v), whereas Koger et al. (2007) reported barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] injury doubled as SVWC content increased from 11 to 49% (v/v) after bispyribac application (22.5 g ai ha^{-1}).

Research to date has shown that indaziflam can move downward in the soil profile and that soil physical and chemical properties may affect plant uptake and subsequent injury. Information on the effect of soil properties on mobility and bioavailability is needed to improve land managers' ability to identify conditions prone to increased indaziflam–soil bioavailability and adjust application practices accordingly to reduce potential injury. The objective of this research was to combine edaphic and plant stress components from previous indaziflam injury-related research to evaluate the effect of SOMC and SVWC on indaziflam–soil movement/bioavailability after application at various rates and timings.

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Table 1. Soil volumetric water content (SVWC) ranges and soil organic matter content (SOMC) pre– and post–indaziflam application (PrIA and PoIA) from 0 to 15 cm depth.[a]

	PrIA SVWC[b]				PoIA SVWC[c]						
	Low		High		Low		High			SOMC	
SOMC, season	Min	Max	Min	Max	Min	Max	Min	Max	Depth	Low	High
	% (cm^3/cm^3)								cm	% (wt/wt)	
Low, Fall	7	15	11	35	6	15	10	39	0 to 2.5	1.4	5.5
Low, Spring	5	14	9	28	5	12	14	36	2.5 to 5	1.1	4.2
High, Fall	12	30	19	32	7	26	19	35	5 to 10	1.2	1.7
High, Spring	14	28	22	28	11	24	18	31	10 to 15	1.1	1.5

[a] Abbreviations: Min, minimum; Max, maximum.

[b] SVWC measurements taken at a 10 cm depth in non–indaziflam-treated plots with WaterScout SM 100 Soil Moisture Sensors® (Spectrum Technologies Incorporated, Aurora, IL).

[c] Excludes recordings for 24 h after irrigation delivered to all plots immediately after indaziflam application (0.6 cm H_2O) and 3 wk after treatment (2.5 cm H_2O).

Materials and Methods

The objective of this research was completed by treating field plots of varying edaphic conditions with indaziflam at various rates and timing and then collecting soil cores for a subsequent greenhouse bioassay experiment. A summary of field conditions and research efforts is provided below, with detailed information regarding field plot establishment and management, experimental design, data collection, and treatment effects on hybrid bermudagrass growth provided in Jeffries and Gannon (2016). After soil core collection, a greenhouse bioassay experiment commenced to measure indaziflam–soil bioavailability.

Field Summary. Field research (Sandhills Research Station, Jackson Springs, NC) was initiated September 23, 2013, and October 1, 2014, to evaluate the effect of SOMC and SVWC on indaziflam–soil bioavailability after indaziflam application(s). Experiments were conducted on established and root-compromised 'Tifway 419' hybrid bermudagrass grown on a Candor sand (sandy, kaolinitic, thermic Grossarenic Kandiudults; 92, 4, and 4% sand, silt, and clay, respectively) with pH 5.2. Because of indaziflam's mode of action and propensity for root uptake, current label wording states application in areas with undeveloped or weakened turfgrass root systems should be avoided (Anonymous 2015). To quantify relationships between indaziflam–soil bioavailability and hybrid bermudagrass growth in differing root system conditions, roots were shortened with a sod cutter (Ryan™ Jr. Sod Cutter 12, Schiller Grounds Care, Johnson Creek, WI) in a strip (50 cm wide by 5 cm deep) within each plot 3 wk before trial initiation. After cutting, the sod was left in place, rolled once, and irrigated twice daily (1.25 cm H_2O $cycle^{-1}$) for 1 wk. At the end of the 1-wk period, pre-indaziflam application (PrIA) SVWC adjustments (to be described) were initiated. Root-compromised strips within each plot were treated as unique areas with respect to data collection. Comparatively, average root length at trial initiation measured 12 cm in established hybrid bermudagrass (i.e., not subjected to root sod cutting).

Indaziflam (Specticle FLO®, Bayer Environmental Science, Research Triangle Park, NC) was applied (50 or 100 g ha^{-1}) at fall-only, fall plus spring, and spring-only timings to plots in a factorial arrangement of SOMC, PrIA SVWC, and post-indaziflam application (PoIA) SVWC. The 100 g ha^{-1} rate (maximum annual application rate) evaluated in the presented research exceeds the currently labeled maximum single application rate (55 g ha^{-1}); however, the authors deemed its inclusion necessary to elucidate confidently the effect of SOMC and SVWC on indaziflam–soil bioavailability. Fall applications were conducted on October 14 and 22 in 2013 and 2014, respectively, whereas spring applications were conducted on March 15 and 18 in 2014 and 2015, respectively. Lastly, spring-only indaziflam application was only evaluated in experimental run 2 because of a space deficiency in run 1.

Within each edaphic variable, "low" and "high" levels were evaluated (Table 1). To evaluate varying SOMC, areas within the trial confinements were stripped of vegetation, tilled, and sprigged, and turfgrass were allowed to establish for 5 mo (> 95% visual hybrid bermudagrass cover at time of trial initiation). Low SOMC at 0 to 2.5, 2.5 to 5,

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5 to 10, and 10 to 15 cm soil depths were 1.4, 1.1, 1.2, and 1.1% (wt/wt), respectively, whereas high SOMC contents were 5.5, 4.2, 1.7, and 1.5% (wt/wt) at the respective depths. SVWC was adjusted for 2-wk PrIA and 6-wk PoIA periods by covering low SVWC plots with tarps (White Poly Tarps, Tarps Plus, Weir, TX) during irrigation and precipitation events. All plots were irrigated immediately after application (0.6 cm H_2O) to conform with label recommendations for acceptable weed control (Anonymous 2015). PoIA low SVWC plots only received water inputs once at 3 WAT (2.5 cm H_2O), whereas PoIA high SVWC plots were irrigated to supplement precipitation to deliver 2.5 cm H_2O wk^{-1}. SVWC was adjusted identically for fall and spring indaziflam applications.

Greenhouse Bioassay. A greenhouse bioassay experiment (North Carolina State University Method Road Greenhouse Facilities, Raleigh, NC) was conducted to quantify indaziflam–soil bioavailability in the soil profile. At 10 and 14 WAT in fall and spring, one core (6.3 cm diam by 15 cm depth) was harvested from each plot and frozen (−12 C) in a plastic liner until bioassay initiation. Fall sampling included core collection from the fall-only treatment, whereas spring sampling included collection from fall plus spring and spring-only application timings. At experiment initiation, the core was allowed to thaw for 30 min, and the plastic liner was split lengthwise to expose a 4-cm section of the soil profile. Exposed soil was then removed with a putty knife to create a flat surface to seed 'Carly' perennial ryegrass at 98 g m^{-2}. All core/seed preparation techniques were performed from the 15-cm depth toward the soil surface to prevent indaziflam contamination to deeper depths. Perennial ryegrass was selected as the bioindicator species based on previous research confirming that the cool-season species was suitable to quantify indaziflam–soil bioavailability (Jhala et al. 2012). Perennial ryegrass was grown in a 29/23 C day/night cycle with supplemental lighting (350 µmol m^{-2} s^{-1}) to provide a 14-h day length. Cores were irrigated twice daily with overhead irrigation and fertilized 0 and 14 d after seeding at 1.2 g N–P–K m^{-2} (Peters Professional 20–20–20 Water Soluble Fertilizer, Scotts-Sierra Horticultural Products Company, Marysville, OH).

Data Collection. After a 28-d growing period, all perennial ryegrass foliage above the soil surface was harvested at 0 to 2.5, 2.5 to 5, 5 to 7.5, 7.5 to 10, and 10 to 15 cm depths from the field plot soil surface and fresh mass was recorded. Foliage was then oven-dried (70 C) for 7 d, and dry mass was recorded. Hybrid bermudagrass cover was visually estimated on a 0 (no cover) to 100% (complete cover) scale at bioassay core collection dates. Perennial ryegrass foliage mass and hybrid bermudagrass cover data were converted to percent reduction relative to the respective nontreated within a replicate using the equation

$$\%\ \text{reduction} = \{[(NT - T)/NT] \times 100\}$$

where NT and T are from a nontreated and treated core or plot, respectively. Turfgrass canopy spectral reflectance measurements were also taken (Crop Circle Model ACS-210®, Holland Scientific, Incorporated, Lincoln, NE) to calculate an average normalized difference vegetation index (NDVI) per plot. To reduce variation, reflectance measurements were taken between 12:00 P.M. and 4:00 P.M. Eastern Standard Time on days with $\leq$ 40% cloud cover (Chang et al. 2005).

Experimental Design and Statistical Analyses. Three replications of a factorial treatment arrangement of indaziflam application rate (50 or 100 g ha^{-1}), indaziflam application timing (fall-only or fall plus spring in both runs; spring-only in second run), SOMC (low or high), PrIA SVWC (low or high), and PoIA SVWC (low or high) were evaluated in a randomized complete block design. Bioassay data were subjected to ANOVA ($P = 0.05$) using mixed models methodology (Statistical Analysis Software—Version 9.2®, SAS Institute, Cary, NC). Experimental run, SOMC, PrIA and PoIA SVWC, and indaziflam application rate and timing were considered fixed effects. Main effects and their interactions are presented accordingly, with precedent given to interactions of increasing magnitude (Steel et al. 1997). Means were separated according to Fisher's protected LSD test ($\alpha = 0.05$), and Pearson's correlation coefficients were determined to quantify the relationships between indaziflam–soil bioavailability and hybrid bermudagrass growth characterized via cover reduction and NDVI.

Results and Discussion

ANOVA did not reveal a significant main effect or interactions, including experimental run; therefore, data were pooled and presented over runs. The overall statistical analysis revealed similar results between fresh and oven-dried perennial ryegrass

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Table 2. Soil organic matter content-by-indaziflam application season and –by-indaziflam application rate interactions on indaziflam-soil bioavailability at 0 to 2.5 cm depth.[a,b]

	10 WAT			14 WAT				
	Season			Season			Rate	
SOMC	F	F+S	S[c]	F	F+S	S	50 g ha^{-1}	100 g ha^{-1} [d]
	% fresh mass reduction[e]							
Low	84	93	87	84	97	89	64	94
High	46	76	51	37	74	55	46	87
$LSD_{0.05}$		15			25		16	

[a] Abbreviations: WAT, weeks after treatment; SOMC, soil organic matter content; F, fall-only; F+S, fall plus spring; S, spring-only.

[b] Data pooled over experimental runs.

[c] Spring-only indaziflam applications evaluated only in experimental run 2.

[d] The 100 g ha^{-1} rate exceeds currently registered maximum single application rate.

[e] Percent fresh mass reduction = $\{[(NT - T)/NT] \times 100\}$, where NT and T are mass from cores collected in nontreated and treated plots, respectively, within a replicate.

mass reduction data, with fresh mass providing greater clarity on the effect(s) of evaluated edaphic conditions and indaziflam applications. Additionally, significant effects of the evaluated variables were only detected from the 0 to 7.5 cm soil depth across fresh and oven-dried data. Therefore, only fresh mass data to a 7.5 cm depth are presented. Significant interactions generally increased in complexity as soil depth increased. Data are presented depthwise from the soil surface, and indaziflam application and edaphic variables that increased indaziflam–soil bioavailability are discussed.

Indaziflam-Soil Bioavailability. *0 to 2.5 cm Soil Depth.* ANOVA revealed SOMC–by–indaziflam application season and SOMC–by–indaziflam application rate interactions on indaziflam–soil bioavailability at 0 to 2.5 cm depth from cores collected 10 and 14 WAT and will be discussed in this order. At 10 WAT, SOMC affected indaziflam–soil bioavailability at all application timings, with perennial ryegrass fresh mass reduction ranging from 84 to 93% and 46 to 76% from low and high SOMC, respectively (Table 2). Within low SOMC, no differences were detected among application seasons (84 to 93% reduction), whereas high SOMC buffered bioavailability from one to two applications, as fall-only and spring-only indaziflam application caused less perennial ryegrass mass reduction (46 and 51%) than fall plus spring application (76%).

At 14 WAT, similar trends were observed. Excluding the fall plus spring application, low SOMC caused greater perennial ryegrass mass reduction (84 to 89%) than high SOMC (37 to 55%; Table 2). No differences were detected across application timings in perennial ryegrass mass reduction in low SOMC (84 to 97%), whereas fall plus spring application in high SOMC doubled bioavailability (74%) compared with fall-only application (37%). Spring-only application did not differ from fall plus spring application in high SOMC. Increasing application rate from 50 to 100 g ha^{-1} increased indaziflam–soil bioavailability 30 and 41% in low and high SOMC, respectively. Indaziflam–soil bioavailability only differed between low (64%) and high (46%) SOMC at 50 g ha^{-1}.

2.5 to 5 cm Soil Depth. Analysis of variance revealed SOMC-by-PoIA SVWC–by–indaziflam application season interactions on indaziflam–soil bioavailability at 2.5 to 5 cm depth from cores collected 10 and 14 WAT. At 10 WAT, SOMC affected bioavailability at all application timings, with perennial ryegrass fresh mass reduction ranging from 61 to 83% and –3 to 37% from low and high SOMC, respectively (Table 3). PoIA SVWC did not affect indaziflam–soil bioavailability in low SOMC, with all perennial ryegrass mass reductions ranging from 61 to 83%; however, increasing SVWC in high SOMC increased bioavailability of spring indaziflam applications. Fall plus spring and spring-only indaziflam application increased perennial ryegrass mass reduction 23 to 28%, respectively, when comparing PoIA low to high SVWC. Single indaziflam applications did not affect bioavailability at PoIA low SVWC, whereas fall plus spring application caused greater perennial ryegrass mass reduction (37%) than fall-only application (18%). At 14 WAT, trends regarding SOMC and PoIA SVWC agreed with data collected 10 WAT. In general, decreasing SOMC and increasing PoIA SVWC increased indaziflam–soil bioavailability. Indaziflam application season caused greater perennial ryegrass mass reduction from fall plus spring (63%) and spring-only (58%) than fall-only (29%) indaziflam application in low SOMC–PoIA low SVWC, whereas differences were not detected between seasons in high SOMC.

ANOVA revealed SOMC–by–PoIA SVWC–by–indaziflam application rate interaction on indaziflam–soil bioavailability at 2.5 to 5 cm depth from

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Table 3. Soil organic matter content-by-post-indaziflam application soil volumetric water content-by-indaziflam application season interaction on indaziflam-soil bioavailability at 2.5 to 5 cm depth.[a]

	10 WAT						14 WAT					
	PoIA low SVWC			PoIA high SVWC			PoIA low SVWC			PoIA high SVWC		
SOMC	F	F+S	S[c]	F	F+S	S	F	F+S	S	F	F+S	S
	% fresh mass reduction[d]											
Low	63	67	61	63	83	71	29	63	58	63	76	84
High	5	14	−3[e]	18	37	25	10	23	5	27	44	26
$LSD_{0.05}$	17						20					

[a] Abbreviations: WAT, weeks after treatment; PoIA, post–indaziflam application; SVWC, soil volumetric water content; SOMC, soil organic matter content; F, fall-only; F+S, fall plus spring; S, spring-only.

[b] Data pooled over experimental runs.

[c] Spring-only indaziflam applications evaluated only in experimental run 2.

[d] Percent fresh mass reduction = {[(*NT* − *T*)/*NT*] × 100}, where *NT* and *T* are mass from cores collected in nontreated and treated plots, respectively, within a replicate.

[e] Negative reduction values indicate an increase in perennial ryegrass fresh mass relative to the nontreated.

cores collected 10 WAT. SOMC affected bioavailability at all application rates, with perennial ryegrass fresh mass reduction ranging from 48 to 78% and 3 to 37% from low and high SOMC, respectively (Table 4). Within low SOMC, increasing PoIA SVWC increased indaziflam–soil bioavailability at both rates, as perennial ryegrass mass reduction increased 18 and 22% after 50 and 100 g ha^{-1}, respectively, in PoIA high compared with low SVWC. Increasing PoIA SVWC also affected increased indaziflam–soil bioavailability 22% in high SOMC at 100 g ha^{-1} when comparing PoIA low to high SVWC. Within PoIA SVWC, increasing application rate increased indaziflam–soil bioavailability only within high SOMC–PoIA high SVWC, as perennial ryegrass mass reduction increased 21%. Although statistical separation of this interaction is not permissible at 14 WAT, general trends agree with data collected at 10 WAT.

5 to 7.5 cm Soil Depth. ANOVA revealed SOMC–by–PoIA SVWC interactions on indaziflam–soil bioavailability at 5 to 7.5 cm depth from cores collected 10 and 14 WAT. At both collection timings, low SOMC–PoIA high SVWC caused greater perennial ryegrass mass reduction (37 to 40%) compared with all other SOMC–-SVWC combinations (–3 to 12%; Table 5). Additionally, no differences were detected in perennial ryegrass mass reduction across SVWC within high SOMC (–3 to 4% and 5 to 12% at 10 and 14 WAT, respectively), or across PoIA low SVWC within low SOMC (–3 to 7% and 5 to 6% at 10 and 14 WAT, respectively).

Table 4. Soil organic matter content-by-post-indaziflam application soil volumetric water content-by-indaziflam application rate interaction on indaziflam-soil bioavailability at 2.5 to 5 cm depth.[a,b]

	10 WAT				14 WAT			
	PoIA low SVWC		PoIA high SVWC		PoIA low SVWC		PoIA high SVWC	
SOMC	50 g ha^{-1}	100 g ha^{-1} [c]	50 g ha^{-1}	100 g ha^{-1}	50 g ha^{-1}	100 g ha^{-1}	50 g ha^{-1}	100 g ha^{-1}
	% fresh mass reduction[d]							
Low	48	56	66	78	41	58	69	80
High	3	7	16	37	8	16	26	39
$LSD_{0.05}$	16				NS			

[a] Abbreviations: WAT, weeks after treatment; PoIA, post–indaziflam application; SVWC, soil volumetric water content; SOMC, soil organic matter content; NS, not significant.

[b] Data pooled over experimental runs.

[c] The 100 g ha^{-1} rate exceeds currently registered maximum single application rate.

[d] Percent fresh mass reduction = {[(*NT* − *T*)/*NT*] × 100}, where *NT* and *T* are mass from cores collected in nontreated and treated plots, respectively, within a replicate.

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Table 5. Soil organic matter content-by-post-indaziflam application soil volumetric water content interactions on indaziflam-soil bioavailability at 5 to 7.5 cm depth.[a,b]

	10 WAT		14 WAT	
SOMC	PoIA low SVWC	PoIA high SVWC	PoIA low SVWC	PoIA high SVWC
	% fresh mass reduction[c]			
Low	7	40	6	37
High	−3[d]	4	5	12
$LSD_{0.05}$	14		15	

[a] Abbreviations: WAT, weeks after treatment; SOMC, soil organic matter content; PoIA, post–indaziflam application; SVWC, soil volumetric water content.

[b] Data pooled over experimental runs.

[c] Percent fresh mass reduction = $\{[(NT - T)/NT] \times 100\}$, where NT and T are mass from cores collected in nontreated and treated plots, respectively, within a replicate.

[d] Negative reduction values indicate an increase in perennial ryegrass fresh mass relative to the nontreated.

Summary. Bioassay results from the present research generally agree with Jhala et al. (2012), in that increasing moisture inputs increased indaziflam–soil bioavailability throughout the soil profile; however, the authors reported 100% perennial ryegrass mortality to a 30 cm depth, which was only observed to a 5 cm depth in the presented research. Discrepancies between results may be explained by indaziflam application to differing sites (established turfgrass vs. bare soil), higher SOMC (soil surface = 1.4 and 5.5% [wt/wt] vs. < 0.5% [wt/wt]), lower carrier volume (304 vs. 6,368 L ha^{-1}), and differing perennial ryegrass seeding intervals after application (10 and 14 wk vs. 18 h). Furthermore, indaziflam was exposed to 5 to 15 cm H_2O shortly after application in the Jhala et al. (2012) research compared with 0.6 cm H_2O in the present research, which would have created a more favorable scenario for downward indaziflam movement. Overall, bioassay results from the present research agree with previous reports of increasing herbicide–soil bioavailability with decreasing SOMC and increasing SVWC (Koger et al. 2007; Pätzold and Brümmer 2003; Song et al. 2010; Sun et al. 2012).

Overall, indaziflam application at fall-only or spring-only timings reduced perennial ryegrass mass similarly, and less than at fall plus spring timings at 10 WAT. This was also true within high SOMC at 14 WAT and low SOMC at 0 to 2.5 cm depth; however, spring-only application decreased perennial ryegrass mass more than fall-only at 2.5 to 5 cm depth. Averaged over low SOMC–PoIA SVWC at 14 WAT, fall-only application reduced perennial ryegrass mass 46% compared with 71% from spring-only application at 2.5 to 5 cm depth (Table 3). This may be due in part to differing rainfall amounts between fall (18.8 and 12.5 cm H_2O in runs 1 and 2, respectively) and spring (2 and 10 cm H_2O in runs 1 and 2, respectively) timings from 10 to 14 WAT, with fall providing more ideal conditions for downward indaziflam movement in the soil profile. Although statistical separation of main effects or interactions was not possible at the 7.5 to 10 cm depth, fall indaziflam applications were required to reduce perennial ryegrass mass 10% (data not shown), which supports the idea that increased rainfall inputs in fall to winter moved indaziflam to deeper soil depths compared with spring to summer.

Increasing indaziflam application rate from 50 to 100 g ha^{-1} generally increased indaziflam–soil bioavailability proportionally in high SOMC; however, perennial ryegrass mass reductions did not respond similarly in low SOMC. Averaged over low SOMC–PoIA SVWC at 10 WAT in the 2.5 to 5 cm depth, perennial ryegrass mass reduction only increased 10% when indaziflam rate increased from 50 to 100 g ha^{-1} (Table 4). Jhala et al. (2012) reported similar findings from indaziflam application at 73 and 145 g ha^{-1}, which increased perennial ryegrass mortality 20% over three simulated rainfall volumes. This may be due in part to downward movement in the profile from low indaziflam sorption affinity, which could decrease soil concentrations below perennial ryegrass sensitivity thresholds.

Correlations of Indaziflam–Soil Bioavailability and Hybrid Bermudagrass Growth. Pearson's correlation coefficients were determined to quantify the relationship between indaziflam–soil bioavailability and hybrid bermudagrass growth characterized by cover reduction and NDVI. At 10 WAST, indaziflam–soil bioavailability was poorly to moderately, positively correlated at 0 to 2.5, 2.5 to 5, 5 to 7.5, 0 to 5, and 0 to 10 cm depths with visual cover estimates of established and root-compromised hybrid bermudagrass, indicating bioavailability increased with increasing cover reduction (Table 6). Correlations were highest for indaziflam–soil bioavailability at 0 to 10 cm depth across established ($r = 0.41$; $P < 0.001$) and 2.5 to 5 cm depths for root-compromised ($r = 0.48$; $P < 0.001$) hybrid bermudagrass. Indaziflam–soil bioavailability was poorly correlated with cover reductions at 5 to 7.5, 7.5 to 10, and 10 to 15 cm

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Table 6. Pearson correlation coefficients for the relationships between indaziflam–soil bioavailability with established and root-compromised hybrid bermudagrass growth characterized via cover reduction and NDVI, 10 and 14 wk after spring treatment.[a–f]

	10 WAST			14 WAST		
	Estab		Comp	Estab		Comp
Depth[g]	CR	NDVI	CR	CR	NDVI	CR
cm	%		%	%		%
0–2.5	0.29*	−0.11	0.34*	0.39*	−0.44*	0.52*
0–5	0.37*	−0.12	0.45*	0.45*	−0.55*	0.59*
0–10	0.41*	−0.16***	0.46*	0.42*	−0.50*	0.56*
2.5–5	0.39*	−0.17**	0.48*	0.42*	−0.55*	0.57*
5–7.5	0.27**	−0.05	0.26**	0.21**	−0.28*	0.24**
7.5–10	0.13***	−0.14***	0.16***	0.12	−0.05	0.14
10–15	0.11	−0.11	0.15	0.08	−0.07	0.13

[a] Abbreviations: NDVI, normalized difference vegetation index (0–1); WAST, weeks after spring treatment; Estab, established; Comp, root-compromised; CR, cover reduction.

[b] Indaziflam–soil bioavailability quantified via fresh perennial ryegrass foliage mass reduction in soil cores collected from field plots.

[c] Roots shortened (5 cm) 3 wk before fall indaziflam application.

[d] Visual cover estimated on a 0 (no cover) to 100% (complete cover) scale.

[e] NDVI = $[(R_{880} - R_{590})/(R_{880} + R_{590})]$, where R_{880} and R_{590} are reflectance at 880 and 590 nm, respectively.

[f] Percent fresh mass and cover reduction = $\{[(NT - T)/NT] \times 100\}$, where NT and T are mass or cover from nontreated and treated core plots, respectively, within a replicate.

[g] Percent perennial ryegrass fresh mass reduction at a respective depth increment.

* Significant at $P < 0.001$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.05$.

depths, which is due to relatively low perennial ryegrass mass reductions at these depths. Additionally, all depths were weakly correlated with NDVI, suggesting spectral reflectance measurements were a poor indicator of indaziflam–soil bioavailability at 10 WAST.

Excluding depths where appreciable perennial ryegrass biomass reductions were not observed (5 to 7.5, 7.5 to 10, and 10 to 15 cm), correlation strength improved from 10 to 14 WAST. At 14 WAST, indaziflam–soil bioavailability was moderately positively correlated at 0 to 2.5, 2.5 to 5, 0 to 5, and 0 to 10 cm depths, with visual cover estimates of established ($r = 0.39$ to 0.45; $P < 0.001$) and root-compromised ($r = 0.52$ to 59; $P < 0.001$) hybrid bermudagrass, and moderately negatively correlated at the same depths with NDVI ($r = -0.44$ to -0.55; $P < 0.001$) (Table 6). Negative relationship indicates that increasing indaziflam–soil bioavailability caused decreased NDVI. Decreasing NDVI can indicate increasing turfgrass stress associated with various abiotic and biotic factors (Settle et al. 2007).

Research Implications. Current wording on the indaziflam turfgrass label informs the end user that coarse or sandy soils (> 80% sand or > 20% gravel) may allow for downward movement in the profile, with which the present research agrees given the sand content evaluated (92%). Results from this research suggest that, in addition to soil texture, SOMC also affects downward indaziflam movement in the profile. Across 0 to 7.5 cm soil depths, low SOMC consistently allowed for greater indaziflam–soil bioavailability than high SOMC, suggesting land managers should consider this edaphic variable before use in potentially injurious conditions. Coupling low SOMC with increased PoIA SVWC also had a pronounced affect on indaziflam–soil bioavailability, as low SOMC–PoIA high SVWC was the only SOMC–SVWC combination that significantly reduced perennial ryegrass mass at the 5 to 7.5 cm depth. Because of indaziflam root absorption, climatic and topographic conditions favoring prolonged PoIA high SVWC should be considered by end users before use in coarse soils with low SOMC.

In addition to the wording pertaining to problematic soil textures on the indaziflam turfgrass label, end users are also informed of numerous abiotic and biotic turfgrass stressors that could exacerbate injury. Additionally, sequential applications at reduced rates are recommended in this scenario. Data from this research support both statements, because moderate correlation strength between indaziflam–soil bioavailability and established hybrid bermudagrass cover reduction improved when compared with root-compromised cover reduction, which growth was adversely affected more in this stressed setting. Although reductions were not always proportionate to the indaziflam application rate adjustment, decreasing rate consistently reduced soil bioavailability. Sequential indaziflam applications at reduced rates may allow for wider use of this PRE herbicide that provides an alternative mode of action for land managers; however, additional research is required to evaluate lower, more applicable rates to ensure adequate weed control is acquired.

In conclusion, this bioassay experiment provided data to supplement previous research efforts

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investigating causal factors of adverse effects on desirable plant growth. Results from this research indirectly agree with preceding efforts, in that edaphic conditions promoting indaziflam–soil bioavailability aligned with field data showing increased adverse effects on plant growth. Future research should evaluate the effect of PoIA SVWC at longer intervals and in finer textured soils and novel formulations on indaziflam–soil bioavailability.

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