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Leaching of Indaziflam Applied at Two Rates Under Different Rainfall Situations in Florida Candler Soil

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Abstract Indaziflam {*N*-[(1R, 2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1RS)-1fluoroethyl]-1,3,5-triazine-2,4-diamine} is a new pre-emergence herbicide recently registered for a broad spectrum weed control in Florida citrus. Experiments were conducted to evaluate leaching of indaziflam applied at 73 and 145 g ai ha^{-1} in Florida Candler soil under simulated rainfall of 5, 10, and 15 cm ha^{-1}. Indaziflam leached the least (12.6 ± 0.6 cm) when applied at 73 g ai ha^{-1} under 5 cm ha^{-1} rainfall. Indaziflam leached furthest (30.2 ± 0.9 cm) when applied at 145 g ai ha^{-1} under 15 cm ha^{-1} rainfall. The visual control ratings of a bio-indicator species ryegrass (*Lolium multiflorum* L.) was 97% at 15 cm ha^{-1} rainfall when indaziflam applied at 145 g ai ha^{-1} in the 26 to 30 cm horizon indicating the maximum movement and activity of indaziflam. A dose response experiment was conducted to determine the sensitivity of ryegrass to various doses of indaziflam that confirmed that application of indaziflam at 29.20 g ai ha^{-1} was sufficient to prevent germination of ryegrass. There was no mortality of ryegrass plants beyond the 30 cm and the biomass of ryegrass was comparable with untreated control indicating that indaziflam did not leach beyond this distance even under 15 cm ha^{-1} rainfall.

Keywords Contamination · Groundwater · Herbicides · Sandy soil · Soil applied · Weed control

Florida is the largest producer of citrus (*Citrus* spp.) in the United States [United States Department of Agriculture (USDA) USDA 2006]. In 2010, citrus was grown on more than 223,000 ha in Florida (USDA 2010a) with the production of >159 million boxes (USDA 2010b). Weed management is a challenge for citrus growers in Florida in order to reduce weed competition with trees as well as to minimize weed interference with horticultural operations (Futch and Singh 2007). Among various methods of weed control, herbicides are an important choice commonly adopted by citrus growers either as strip applications within the crop row or as broadcast applications to the grove floor (Sharma and Singh 2007a).

Pre-emergence herbicides, also known as soil applied or residual herbicides are applied to the soil surface for weed control in Florida citrus. Ideally, they should be followed by irrigation or rainfall, so that moisture carries herbicide into the soil. Some commonly used pre-emergence herbicides in Florida citrus include bromacil (5-bromo-3-sec-butyl-6- methyluracil), diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], simazine [2-chloro-4,6-bis(ethylamino)-s-triazine], pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine)], norflurazon {4-chloro-5-(methylamino)-2-[3(trifluoromethyl)phenyl]-3(2H)-pyridazinone} etc. (Futch and Singh 2010).

Indaziflam (Alion$^{\text{TM}}$; Bayer CropScience, Research Triangle Park, NC, USA) is a new alkylazine herbicide being developed for pre-emergence control of monocot and dicot weeds in commercial crop production, turf, commercial non-crop areas, field grown ornamentals, commercial nurseries, landscape plantings, and forestry sites. The labeled rate of indaziflam ranges from 73 to 145 g ai ha^{-1} (5.0 to 6.5 fl oz acre^{-1}) in a single application with a maximum cumulative annual amount of 150 g ai ha^{-1} (10.3 fl oz acre^{-1}) in Florida citrus (Anonymous 2011). In field experiments conducted in Florida, indaziflam provided 3–5 months of residual weed control

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in citrus depending on weather conditions and weed pressure (Singh et al. 2011). Thus, indaziflam could be an additional herbicide option for citrus growers for pre-emergence weed control.

Frequent rainfall and sandy soil in citrus growing regions in Florida provide the best opportunity for herbicide leaching and the potential for groundwater contamination. The annual rainfall in Florida ranges from 120 to 150 cm (47–59 inches); however, the amount that falls in any individual year may vary from 94 to 254 cm (37–100 inches) (Davies and Jackson 2009). In 2010, rainfall in central Florida was in the range of 100–117 cm (40–46 inches) (Florida Forest Service 2011).When herbicides leach beyond the zone of weed seed germination, they may damage citrus tree roots, result in poor weed control, and also create environmental problems such as groundwater contamination. For example, bromacil is a commonly used, soil applied herbicide in citrus. Because of its leaching and groundwater contamination, it was banned for use in Florida's deep sandy Entisols (Fishel 2011); however, bromacil is still used in some citrus production areas in Florida.

The Environmental Hazards Assessment Program of the California Department of Food and Agriculture (CDFA) conducted a study to assess the movement of simazine and diuron through soil in citrus groves. Low levels of one or both of these herbicides were found in seven of twelve sampled wells (Welling et al. 1986). Leaching of herbicides has been reported not only in sandy soils, but in other soils and cropping systems as well. For example, atrazine [2-chloro-4-(ethylamine)-6-(isopropylamine)-1,3,5-triazine] is an herbicide that selectively controls broadleaf weeds in maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and many other crops. The relative mobility of atrazine and five atrazine degradates were determined in both surface and subsurface soils from five locations in Iowa and the results suggested that deethylatrazine was the most mobile compound followed by atrazine (Kruger et al. 1996). Similarly, alachlor [2-chloro-2′-6′-diethyl-*N*-(methoxymethyl)-acetanilide] and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) has been detected in well water from several areas in the United States and the source of these herbicides were from the field applications in number of crops in different soil types (Anonymous 1991; Chesters et al. 1989; Thurman et al. 1990). Therefore, it is advisable to study the movement and leaching of new herbicide active ingredients, before their repeated applications and accumulation in field soils.

Several screening procedures have been developed to rank soil applied herbicides based on their leaching behavior in the soil profile (Forney et al. 2000; Mueller and Banks 1991; Weber et al. 1986). In spite of rapid developments in analytical methods (Kruger et al. 1996), bioassay remains an important tool for qualitative and quantitative determination of herbicide leaching (Nelson and Penner 2007). The use of soil columns as a technique to simulate herbicide movement has been reported (Blumhorst 1996; Futch and Singh 1999; Weber et al. 1986). Bioassay procedures are usually more economical, less difficult to perform, take less time, and do not require expensive equipment compared to chemical analytical methods (Mueller and Banks 1991).

Indaziflam is a soil applied herbicide and with its new chemistry and mode of action, it might be useful for broad spectrum, pre-emergence weed control in Florida citrus; however, indaziflam may impact surface water quality due to runoff in rain water (Anonymous 2011). In addition, indaziflam has properties and characteristics associated with chemicals detected in groundwater (USEPA 2011). There is no information available on leaching of indaziflam in Candler soil. The objective of this investigation was to evaluate the mobility of indaziflam under different rainfall situations in Florida Candler fine sand.

Materials and Methods

The soil for this experiment was collected from a citrus grove near Davenport, FL. The site had been a citrus grove and has been free from any crop or agricultural operations for more than 15 years. This location was known to be free from any pesticide use for at least 15 years. Soil samples were collected from the top 120 cm profile at different horizons (0–30, 31–60, 61–90, and 91–120 cm). The soil was typical well-drained, fine Chadler sand (Hyperthermic, uncoated Typic Quartzipsamments) (Brown 2003). The soil samples of different depths were stored in separate, open, wooden containers and were air-dried for 2 weeks before use. Two representative soil samples of about 100 g were randomly collected from different soil horizons, packed in paper bags and sent for soil chemical analysis at the Waters Agricultural Laboratories, Inc., Camilla, GA (Table 1).

The experiment was conducted using soil leaching columns under greenhouse conditions at the Citrus Research and Education Center, University of Florida, Lake Alfred, FL. The greenhouse was maintained at day/night temperature of 25/16°C (±0.5°C), 70% (±5%) relative humidity and normal photoperiod. Soil columns were 150 cm long polyvinyl chloride (PVC) pipes with a 10 cm inner diameter. The pipes were cut into halves longitudinally. To prevent preferential flow of herbicides along the soil-column interface, silicone ridges were placed cross-sectional at 15 cm intervals along the inner wall. The halves were sealed using an adhesive tape (Professional HVAC tape, Scotch brand) to form a column, and the bottom end was fitted with a PVC cap. A nylon screen with Whatman filter

Table 1 Analysis of samples collected from different soil horizons of a citrus grove near Davenport, FL

Soil depth[a] (cm)	pH	CEC meq 100 g^{-1}	Sand (%)	Silt (%)	Clay (%)	OM (%)	ENR (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Mg (kg ha^{-1})	Ca (kg ha^{-1})	B (kg ha^{-1})	Zn (kg ha^{-1})
0–30	6.3	2.9	91.6	4.4	4	0.46	8.74	241	13	57	910	0.45	25
31–60	5.8	1.5	89.2	6.4	4.4	0.24	5.16	91	12	30	254	0.34	4.7
61–90	5.6	1.2	91.2	4.8	4.0	0.18	4.15	86	11	25	233	0.22	3.7
91–120	5.2	1.2	93.2	2.4	4.4	0.15	3.25	81	11	17	169	0.22	1.8

CEC cation exchange capacity, *OM* organic matter, *ENR* estimated nitrogen release

[a] The analysis of soil samples was performed at the Waters Agricultural Laboratories, Inc., Camilla, GA

paper # 4 was placed at the bottom of the PVC cap. The soil columns were filled with soil incrementally from the horizons collected from the four depths (0–30, 31–60, 61–90, and 91–120 cm). Soil columns were secured upright and each column was watered to field capacity, and allowed to drain for 15 h.

The experiment was conducted in a randomized complete block design with three replications. Indaziflam was applied at 73 and 145 g ai ha^{-1}. An herbicide solution was prepared in a 5 mL solution in a 20-mL vial, and was shaken vigorously prior to application to the soil column. An untreated control soil column was included for comparison which was applied with 5 cm ha^{-1} rainfall but no indaziflam. An herbicide solution was applied with a small dropper to soil surface in the soil column. Whatman filter paper #4 was placed on surface of the soil column (after application of herbicide inside the column), and then a 1.25-cm layer of silanized-grade glass wool was placed on the surface to ensure proper spread and uniform solution flow of water through the soil column. It is in the intensity of its precipitation that Florida differs from other states in the nation. To simulate a rainfall of 5, 10, and 15 cm ha^{-1}, deionized water was dripped from a siphon system attached to a 1,000-mL Erlenmeyer flask mounted above each soil column over the glass wool. A total six treatments including two rates of indaziflam and three levels of rainfall were compared with untreated control (Table 2). The volume of water to be applied in each soil column was calculated as per the formula,

$$\text{Volume of water for 5 cm ha}^{-1}\text{ rain fall} = \pi r^2 h$$

where, $\pi = 3.1428$; r = radius of the column (5 cm), and h = height of the rainfall (5 cm).

For example, volume of water required per soil column to simulate a rainfall of 5 cm ha^{-1} was 392.5 mL.

The intensity of rainfall, that is, the amount falling in any 24 h period is vary from a trace (less than 0.025 cm) to as much as 41 cm; however, it is very common to have a rainfall of about 2.0 cm h^{-1} in citrus growing area in central Florida (Davies and Jackson 2009). Preliminary experiments were conducted to determine the flow of water (droplets) from a siphon system attached to a 1,000-mL Erlenmeyer flask mounted in a wooden stand. The time was recorded using a stop watch for 15 min and water was collected in a cylinder which was measured with a rain gauge to establish a simulated rainfall of 2.0 cm h^{-1}. During the experiment, the flow rate of water (droplets) was visually monitored with a stop watch to ensure uniform leaching approximately at pre-established rate of 2.0 cm h^{-1}. The soil columns remained intact for 18 h after herbicide and rainfall treatments then were split longitudinally by removing the duct tape from one side and slicing the soil along the center, after removing the PVC cap at the end of the soil column. Using a one-meter ruler,

Table 2 Effects of application rate and amount of rainfall on the depth of indaziflam leaching as indicated by ryegrass bioassay

Indaziflam rate (g ai ha^{-1})	Rainfall (cm ha^{-1})	Depth of indaziflam leaching[a, b] (cm)
Untreated control	5	0 ± 0.0 a
73	5	12.6 ± 0.6 b
73	10	19.5 ± 0.9 d
73	15	23.4 ± 1.0 e
145	5	18.2 ± 1.4 c
145	10	23.3 ± 1.7 e
145	15	30.2 ± 0.9 f
p value	–	<0.0001
Contrast statements[c]		
73 vs. 145 g ai ha^{-1}		*
Rain: 5 vs. 10 cm ha^{-1}		*
Rain: 5 vs. 15 cm ha^{-1}		*
Rain: 10 vs. 15 cm ha^{-1}		*

[a] Mean distance of herbicide moved ±SD (n = 6); The data were $\log_{10}(x + 1)$ transformed for homogenous variance prior to analysis; however, data presented are the means of actual values for comparison

[b] Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference (LSD) test, $p < 0.05$

[c] Orthogonal contrast denoted by an asterisk is significant at $p < 0.05$

three shallow furrows were made on the soil surface with a distance of 2.5 cm between each furrow.

Ryegrass (*Lolium multiflorum* L.) was used as a bio-indicator species and the seeds were planted in a very thick stand in previously prepared furrows, and the seeds were covered with adjacent soil (Futch and Singh 1999; Lavy and Santelman 1986; Sharma and Singh 2007b). The split soil columns were arranged on a wooden bench in the greenhouse and mist-irrigated three times each day. Data were collected for the depth of indaziflam leaching (distance moved) indicated by ryegrass plant mortality or injury symptoms exhibited on the seedlings in soil columns at 15 days of indaziflam treatment. The depth of indaziflam leaching was recorded by measuring the distance from the top of the soil surface to the area where mortality of the ryegrass plants was observed. Percent mortality of ryegrass plants separately from each horizon (0–30, 31–60, 61–90, and 91–120 cm) was recorded based on a 0% to 100% scale with 0% being no death or injury symptoms and 100% being complete mortality of ryegrass plants. Ryegrass plants from each 30 cm horizon (0–30, 31–60, 61–90, and 91–120 cm) were cut from the base of the plant, placed in paper bags, and weighed. The fresh weight of ryegrass, expressed as biomass weight was recorded. The experiment was repeated to confirm the results.

The experiment was also conducted to determine the sensitivity of the bioassay species (ryegrass) to various doses of indaziflam applied pre-emergence. The experiment was conducted in a randomized complete block design with three replications. Twenty ryegrass seeds were planted in the soil collected from the citrus grove (explained previously) from the first horizon (0–30 cm depth) in plastic containers with 11-cm diameter and 5 cm height. The plastic containers were watered and kept overnight under greenhouse conditions. Indaziflam was applied at 1.83, 3.65, 7.30, 14.62, 29.20, 43.82, 58.41, 73.00, 95.20, and 146.00 g ai ha^{-1}. The indaziflam solution was prepared in a 5 mL solution in a 20-mL vial, and was shaken vigorously prior to application to the soil column. An herbicide solution was applied with a small plastic dropper to the soil surface on the next day of planting seeds. An untreated control container was included for comparison. The plastic containers were mist irrigated two times in a day. The germination of ryegrass plants was recorded 10 days after indaziflam treatment. The experiment was repeated with the same procedure.

Data were subjected to analysis of variance (ANOVA) using the statistical analysis software version 9.2 (SAS Institute Inc, Cary, NC, USA). Normality, homogeneity of variance, and interactions of treatments in repeat experiments were tested. In this experiment, treatment by experiment interaction was non-significant, therefore, the data of two experiments were pooled and the combined data were presented. The data of depth of leaching were $\log_{10}(x + 1)$ transformed and the data of percent ryegrass mortality were arc-sine transformed prior to analysis to meet assumptions of variance analysis. However, non-transformed percentages are presented with mean separation based on transformed data. Where the ANOVA indicated that treatment effects were significant, means were separated at $p < 0.05$ lsmeans and adjusted with Fisher's protected least significant difference (LSD) test. In addition, orthogonal contrasts were performed as part of the ANOVA procedure. Specific contrast tested included indaziflam application rates: 73 vs. 145 g ai ha^{-1}; amount of rainfall: 5 vs. 10 cm ha^{-1}; 5 vs. 15 cm ha^{-1}; and 10 vs. 15 cm ha^{-1}. Differences were considered significant when $p < 0.05$.

Results and Discussion

The results of soil chemical analysis suggested that the soil samples collected from different depths from a site near Davenport, FL had >89% sand, <7% silt, <5% clay, and <0.5% organic matter content (Table 1). These are typical characteristics of the Candler fine sand soils in citrus growing regions in Florida (Brown 2003).

Indaziflam application rates and amount of rainfall had a significant impact on depth of leaching. Compared to untreated soil columns, leaching was observed with any rate of indaziflam or amount of rainfall ($p < 0.0001$) (Table 2). The least movement was observed with indaziflam applied at 73 g ai ha^{-1} at the lowest amount of rainfall (5 cm ha^{-1}). In a previous study, leaching of herbicides in soils was governed largely by the amount of rainfall and soil type (Weber and Miller 1989). Similar results were observed in this study; for example, indaziflam applied at 73 g ai ha^{-1} leached 19.5 ± 0.9 cm and 23.4 ± 1.0 cm at 10 and 15 cm ha^{-1} rainfall, respectively (Table 2). The highest leaching was observed when indaziflam was applied at 145 g ai ha^{-1} at 15 cm ha^{-1} rainfall compared to other treatments. Primary soil factors that influence herbicide leaching to the groundwater are organic matter content and texture (Leonard and Knisel 1988). Florida Candler soil has more than 89% sand and is very low in organic matter content (< 0.5%) (Table 1) which might have provided more opportunity for indaziflam leaching.

The results of orthogonal contrasts of indaziflam application rates suggested that there was a positive correlation between indaziflam application rates which has been reflected in more leaching of indaziflam at high rate and vice a versa. The same correlation was observed with amount of rainfall (Table 2) as leaching was increased with increasing amount of rainfall. In a similar experiment, Sharma and Singh (2001) reported positive correlation between amount of rainfall and herbicide leaching. They showed that leaching depth of norflurazon (Solicam^{TM}; Syngenta Crop Protection Inc, Greensboro, NC) increased from 19.6 to 105.4 cm with increasing amount of rainfall from 6.25 to 12.5 cm ha^{-1}.

Visual control ratings of bio-indicator species (ryegrass) taken from the different depths of the soil columns showed that significant mortality of ryegrass occurred in the 0 to 30 cm depth ($p < 0.0001$) (Table 3). Compared to untreated soil columns, 100% mortality of ryegrass was observed in all the treatments in the 0 to 5 and 6 to 10 cm horizon. The mortality was increased with increasing rate of indaziflam and amount of rainfall beyond 15 cm. For example, indaziflam applied at 73 g ai ha^{-1} and 5 cm ha^{-1} rainfall resulted in 0% mortality compared to ≥90% mortality with 10 or 15 cm ha^{-1} rainfall in the 16–20 cm horizon (Table 3). The result of orthogonal contrasts of indaziflam application rates on percent mortality of ryegrass was significant and ryegrass mortality was usually increased with increasing rate of indaziflam in the 16–20 and 21–25 cm horizon. Similar results were observed for the amount of rainfall and percent mortality of ryegrass (Table 3). There was no mortality of ryegrass beyond 30 cm (Table 4). Similar results were observed in previous studies with the highest mortality of ryegrass in the top layer of the soil leaching columns (Futch and Singh 1999; Sharma and Singh 2001). There was no mortality of ryegrass beyond 30 cm in any of the herbicide treatments.

The biomass of ryegrass recorded after 15 days of indaziflam application suggested that compared to untreated soil columns, all the treatments had less biomass in the 0–30 cm horizon (Table 4). The application rate of indaziflam and simulated rain affected ryegrass biomass in the 0–30 cm horizon. Among treated columns, the lowest biomass was recorded when indaziflam was applied at the highest rate and rainfall. By comparing the results of this study with previous leaching studies of soil applied herbicides commonly used in citrus (Futch and Singh 1999; Reddy and Singh 1993; Sharma and Singh 2001; Tan and Singh 1995), indaziflam can be considered as a moderate leaching herbicide. There was no difference in ryegrass biomass among treatments at distance beyond 30 cm suggested that indaziflam leaching was restricted to the 30 cm horizon in this study (Table 4). Result of orthogonal contrasts of indaziflam application rates on biomass was non-significant. The bio-indicator species (ryegrass) was very sensitive to indaziflam that was reflected in a dose response study (Table 5). Indaziflam rate as low as 29.20 g ai ha^{-1} was sufficient to prevent germination of ryegrass suggesting that a dose of at least 29.20 g ai ha^{-1} reached maximum up to 30 cm. With reducing dose of indaziflam, ryegrass germination was increased and up to 80% germination was observed at the dose of 1.83 g ai ha^{-1}. These results suggested that use of ryegrass as a model species to detect indaziflam leaching was appropriate in this study.

After application on the soil surface, the soil applied herbicides undergo several processes including degradation and transport (Vink and Robert 1992). Herbicides with high persistence and a strong sorption rate are likely to remain near the soil surface, increasing the chances of being carried to a stream or lake via surface runoff. In

Table 3 Effect of indaziflam rate and amount of rainfall on percent mortality of ryegrass in the 0–30 cm column

Indaziflam rate (g ai ha^{-1})	Rainfall (cm ha^{-1})	Mortality of ryegrass[a, b] (%)					
		0–5 cm	6–10 cm	11–15 cm	16–20 cm	21–25 cm	26–30 cm
Untreated control	5	0 a	0 a	0 a	0 a	0 a	0 a
73	5	100 b	100 b	50 b	0 a	0 a	0 a
73	10	100 b	100 b	100 c	90 c	0 a	0 a
73	15	100 b	100 b	100 c	100 c	72 b	0 a
145	5	100 b	100 b	100 c	65 b	0 a	0 a
145	10	100 b	100 b	100 c	100 b	68 b	0 a
145	15	100 b	100 b	100 c	100 b	100 c	100 b
Contrast statements[c]							
73 vs. 145 g ai ha^{-1}		NS	NS	*	*	*	*
Rain: 5 vs. 10 cm ha^{-1}		NS	NS	*	*	*	NS
Rain: 5 vs. 15 cm ha^{-1}		NS	NS	*	*	*	*
Rain: 10 vs. 15 cm ha^{-1}		NS	NS	NS	NS	*	*

[a] The data of percent mortality of ryegrass were arc-sine transformed for homogenous variance prior to analysis; however, data presented in this table are the means of actual values for comparison

[b] Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference (LSD) test, $p < 0.05$

[c] Orthogonal contrast denoted by an asterisk (*) is significant and that denoted by NS is non-significant at $p < 0.05$

Table 4 Effect of indaziflam rate and amount of rainfall on percent mortality and biomass of ryegrass in 31–120 cm horizons

Indaziflam rate (g ai ha^{-1})	Rainfall (cm ha^{-1})	Mortality of ryegrass[a, b] (%)			Biomass of ryegrass[a, b] (g)			
		31–60 cm	61–90 cm	91–120 cm	0–30 cm	31–60 cm	61–90 cm	91–120 cm
Untreated control	5	0 a	0 a	0 a	12.8 ± 0.3 a	12.7 ± 0.3 a	12.0 ± 0.6 a	13.2 ± 0.5 a
73	5	0 a	0 a	0 a	6.8 ± 0.6 b	12.6 ± 0.4 a	12.4 ± 0.4 a	13.5 ± 0.3 a
73	10	0 a	0 a	0 a	5.1 ± 2.9 bc	12.4 ± 0.5 a	12.6 ± 0.3 a	13.4 ± 0.3 a
73	15	0 a	0 a	0 a	5.5 ± 5.5 bc	13.0 ± 0.2 a	12.7 ± 0.3 a	13.1 ± 0.6 a
145	5	0 a	0 a	0 a	7.3 ± 0.2 b	12.7 ± 0.3 a	12.6 ± 0.3 a	13.0 ± 0.6 a
145	10	0 a	0 a	0 a	3.3 ± 1.2 c	12.8 ± 0.2 a	12.0 ± 0.6 a	13.4 ± 0.3 a
145	15	0 a	0 a	0 a	0.2 ± 0.0 d	12.5 ± 0.5 a	12.7 ± 0.3 a	13.2 ± 0.5 a
Contrast statements[c]								
73 vs. 145 g ai ha^{-1}		NS	NS	NS	NS	NS	NS	NS
Rain: 5 vs. 10 cm ha^{-1}		NS	NS	NS	NS	NS	NS	NS
Rain: 5 vs. 15 cm ha^{-1}		NS	NS	NS	*	NS	NS	NS
Rain: 10 vs. 15 cm ha^{-1}		NS	NS	NS	NS	NS	NS	NS

[a] The data of percent mortality of ryegrass were arc-sine transformed for homogenous variance prior to analysis; however, data presented in this table are the means ± SD (n = 6) of actual values for comparison

[b] Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference (LSD) test, $p < 0.05$

[c] Orthogonal contrast denoted by an asterisk is significant and that denoted by NS is non-significant at $p < 0.05$

Table 5 Sensitivity of ryegrass to various doses of indaziflam in a dose response study

Indaziflam rate (g ai ha^{-1})	Germination of ryegrass[a, b] (%)
Untreated control	99 ± 1.0 f
1.83	80 ± 1.2 e
3.65	30 ± 1.0 d
7.30	20 ± 0.8 c
14.62	10 ± 0.5 b
29.20	0 ± 0.0 a
43.82	0 ± 0.0 a
58.41	0 ± 0.0 a
73.00	0 ± 0.0 a
95.20	0 ± 0.0 a
146.00	0 ± 0.0 a

[a] The data of germination of ryegrass were arc-sine transformed for homogenous variance prior to analysis; however, data presented in this table are the means ± SD (n = 6) of actual values for comparison

[b] Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference (LSD) test, $p < 0.05$

contrast, herbicides with high persistence and weak sorption rate may be readily leached through the soil and are more likely to contaminate groundwater (Hornsby et al. 1991). For example, the soil-organic carbon sorption coefficient (K_{oc}) value of bromacil is 32 mL g^{-1}, therefore, it is highly mobile especially in fine-textured soils (Buttler et al. 1992). The K_{oc} value of indaziflam is <1,000 mL g^{-1}, so it is expected to be moderately mobile in the soil and moderately persistent in aerobic soil (half-life >150 days). Indaziflam dissipates in the environment primarily through degradation and leaching. Indaziflam metabolites are more mobile than the parent, and were detected in field studies at the deepest depths sampled (105–120 cm) (USEPA 2011). Therefore, indaziflam metabolites have the potential to leach to groundwater. A previous study reported that addition of adjuvants reduced leaching of the soil applied herbicide pendimethalin (Prowl H_2O; BASF, Research Triangle Park, NC, USA) in Florida Candler soil (Sharma and Singh 2007b). Therefore, more research is required to evaluate suitable adjuvant(s) which may reduce leaching of indaziflam.

This is the first report of leaching behavior of indaziflam in sandy soil. Overall results suggested that there was a positive correlation between application rate of indaziflam and amount of rainfall with depth of leaching of indaziflam. There was a limited opportunity where indaziflam leached with the front of the water moving through the column; it might not have been detected with this bioassay. There was no leaching of indaziflam beyond the 30 cm horizon even under high rainfall situation (15 cm ha^{-1}) indicating that the mobility of indaziflam is moderate. More research is required to evaluate leaching of indaziflam with rainfall >15 cm ha^{-1}.

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