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Effects of herbicide management practices on the weed density and richness in dicamba-resistant cropping systems in Indiana

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Research Article

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Abstract

The addition of dicamba as a weed control option in soybean [*Glycine max* (L.) Merr.] is a valuable tool. However, this technology must be utilized with other herbicide sites of action (SOAs) to reduce selection pressure on weed communities and ensure its prolonged usefulness. A long-term trial was conducted for 7 yr in Indiana to evaluate weed community densities and species richness with four levels of dicamba selection pressure in a corn (*Zea mays* L.)–soybean rotation. Monocot densities and richness increased over time in the dicamba-reliant treatment. Dicot densities in the dicamba-reliant treatment declined over time, but dicot richness increased. The soil weed seedbank was affected by the varying herbicide strategies. The dicamba-reliant strategy had greater than 43% higher total weed density than all other treatments, primarily due to having a monocot density that was at least 71% higher than the other treatments. The fully diversified strategy with eight SOAs and residual herbicides used every year had the lowest total weed species richness in the soil seedbank, which supported the in-field observations.

Introduction

Weeds are the most damaging of pests in agronomic production systems, with competition from weeds causing greater yield losses than insects or pathogen pathogens (Oerke 2006). Herbicides are the primary source of control in corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] in the United States (Gianessi and Reigner 2007). Selective herbicides, such as dicamba, a Group 4 herbicide, have been useful for several decades for targeting specific weeds in monocot field crops (Canode and Robocker 1970). Dicamba controls broadleaf weeds within grass crops, as grasses are able to effectively metabolize the herbicide to prevent injury (Chang and Born 1971). With the recent commercialization of dicamba-resistant soybean varieties, the use of dicamba has increased substantially. Approximately 60% of the soybean acres in the United States were dicamba resistant in 2019 (Unglesbee 2019).

Weed management practices impose selection pressures that drive shifts in weed communities. Documented cases of weed shifts due to changes in weed management have occurred as a result of tillage, irrigation systems, herbicide use, and crop rotation (Brim-DeForest et al. 2017; Davis et al. 2009; Johnson and Coble 1986; Johnson et al. 2009; Menalled et al. 2001; Tuesca et al. 2001). Shifts in weed species have also been observed when comparing glyphosate-resistant cropping systems with conventional herbicide systems. Late-emerging weed species have been shown to be more prevalent in cropping systems that use POST herbicide applications (Swanton et al. 2010). Johnson et al. (2009) discussed the concern of weed populations shifting to more problematic and herbicide-resistant weed biotypes that will reduce the usefulness of technologies such as glyphosate-resistant crops.

One of the most prevalent weed shifts in modern history occurred as a result of the wide-scale adoption of glyphosate-resistant corn, soybean, and cotton (*Gossypium hirsutum* L.) (Dill et al. 2008). This led to more than one application per season of glyphosate being applied to many acres with little diversity in herbicide strategies. This process selected for glyphosate-resistant biotypes, which Young (2006) argued would be a negative consequence of this technology. In the absence of an integrated weed management approach, weed shifts from tolerant to resistant species will occur as a result of widespread adoption of dicamba-resistant soybean. Both species richness and evenness were greater when crop rotations were not implemented or were continually planted to glyphosate-resistant traits (Young et al. 2013). A survey of Nebraska farmers in 2017 showed that 20% had planted soybean resistant to dicamba and glyphosate, and that 60% of those used dicamba, glyphosate, or the combination of the two as their only source of POST weed control (Werle et al. 2018). Although research has shown that dicamba can be a valuable tool for controlling problematic weeds (Chahal and Johnson 2012), it is important to use multiple effective SOAs to reduce the selection pressure for dicamba-resistant biotypes (Shergill et al. 2017).

Dicamba-resistant soybean varieties were introduced to be commercially grown before the 2017 growing season and were developed to aid producers in controlling problematic herbicide-resistant broadleaf weeds. There are several herbicide-resistant weed species that present challenges to Indiana growers, including horseweed [*Conyza canadensis* (L.) Cronquist], giant ragweed (*Ambrosia trifida* L.), waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], and Palmer amaranth (*Amaranthus palmeri* S. Watson) (Heap 2020). Glyphosate-resistant *C. canadensis* was detected in anywhere from 15% to 78% of populations across all regions of Indiana more than 12 yr ago (Davis et al. 2008). These herbicide-resistant weeds pose a threat to corn and soybean yield. *Ambrosia trifida* can reduce yields of soybean as much as 52% with densities of only 2 plants 3 m^{-2}, and can reduce corn yields by up to 90% under high *A. trifida* densities (Baysinger and Sims 1991; Harrison et al. 2001). The addition of dicamba as an active ingredient in soybean will provide in-season control options for several glyphosate-resistant broadleaf weed species, as the addition of dicamba to glyphosate increased the control of glyphosate-resistant *A. palmeri, A. tuberculatus*, and C. *canadensis* to at least 95% (Johnson et al. 2010). Byker et al. (2013) found that 900 g ae ha^{-1} glyphosate + 600 g ae ha^{-1} dicamba applied preplant, followed by a POST application of 900 g ha^{-1} of glyphosate + 300 g ha^{-1} of dicamba, resulted in at least 95% *C. canadensis* control in dicamba-resistant soybeans across three locations in Ontario, Canada. However, Spaunhorst and Johnson (2016) reported that utilizing dicamba as a PRE herbicide alone could result in less than 50% control of glyphosate-resistant *A. palmeri*, but when used with metribuzin, control increased more than 30%. It will be important to utilize dicamba-resistant soybeans with multiple other SOAs and residual herbicides in years that both corn and soybean are grown.

Currently there are only two reported species that have evolved resistance to dicamba in the United States (Heap 2020). The two species with reported resistance are kochia [*Bassia scoparia* (L.) A.J. Scott] and prickly lettuce (*Lactuca serriola* L.), both common in small grains production, where crop rotation is minimal and dicamba is applied year after year and auxins are heavily relied upon (Fernandez-Cornejo et al. 2011). Therefore, there is a reasonable concern that dicamba resistance will evolve as auxin-resistant soybean varieties are used on more acreage because of overuse of this SOA for controlling other herbicide-resistant broadleaf weeds. Although dicamba resistance could evolve, a shift toward more tolerant monocot species is likely to occur without application of residual herbicides and would likely be a more immediate concern.

The objective of this research was to identify shifts in the weed community, in terms of both weed density and species richness, in a corn–soybean rotation with varying levels of dicamba selection pressure over 7 yr.

Materials and Methods

Field Sites

A field trial in a corn–soybean rotation was initiated in 2013 and continued through the 2019 growing season. Experiments were conducted at the Throckmorton Purdue Agricultural Center, Lafayette, IN (40.30°N, 86.90°W). Global positioning system coordinates were taken to mark the corners of the trial areas due to the long-term nature of this project to ensure the trials remained in the same location throughout the 7-yr period. Corners of the trial area were additionally marked to ensure trial remained in the same location from year to year. The site was a conventional-till site on a Toronto-Millbrook (fine-silty, mixed, superactive, mesic Udollic Epiaqualfs) complex that was chisel plowed in the fall and disked and field cultivated in the spring. The soil has an organic matter of 2.6%, a pH of 6.1, and a CEC of 10.6 meq 100 g^{-1}. Fertility programs were adjusted based on soil test values. Corn was planted in 2013, 2015, 2017, and 2019 at a rate of 80,000 seeds ha^{-1}, while soybean was planted in 2014, 2016, and 2018 at a rate of 350,000 seeds ha^{-1}. Corn hybrids used had traits that conferred resistance to both glufosinate and glyphosate. Soybean varieties used were resistant to dicamba and glyphosate.

Experimental Design and Herbicide Strategies

The experimental design was a random complete block with six replications. Plot size was 6-m wide and 18-m long. Herbicide strategies were developed to evaluate weed community shifts as SOAs are implemented into a 2-yr corn–soybean cropping system. The four treatments were labeled as follows: (1) dicamba reliant, (2) diversified glyphosate, (3) diversified dicamba, and (4) fully diversified consisting of three, six, seven, and eight SOAs (Table 1). Herbicides were chosen in order to control the problematic weeds. Herbicide applications were made with a 3-m CO_2 -propelled backpack sprayer calibrated to deliver 140 L ha^{-1} at 4.8 km h^{-1}. Flat-fan AIXR11002 nozzles (TeeJet Technologies, 1801 Business Park DR, Springfield, IL 62703) were used to apply treatments that did not contain dicamba, while TTI11003 nozzles (TeeJet Technologies) were used for dicamba applications. Herbicide application dates for each year can be found in Table 2. During corn years, two applications were made to each plot, including a PRE, followed by a POST when weeds were 10- to 15-cm tall or when corn reached 76 cm in height. POST applications were made in mid-June and are hereafter referred to as "early-summer" evaluations. During soybean years a PRE was applied followed by a POST application when weeds were 10 to 15 cm. An early-POST followed by a late-POST application was used in the dicamba-reliant strategy due to the lack of a soil residual herbicide in soybean years.

Field Data Collection

To monitor changes over time in weed density and species richness within plots, two 1-m^2 quadrats were placed 4.5 m from the back and front of each plot and 1 m from plot edges. These quadrats were placed in the same location every year. Weed densities and species richness were recorded before POST herbicide applications. Trials were harvested once crops reached physiological maturity, and grain weight and moisture for reach plot were recorded. Weed density and richness were partitioned into total weed measurements, as well as separated into dicot and monocot categories. Data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS v. 9.4 (SAS, 100 SAS Campus Drive, Cary, NC 27513-2414), with year treated as a repeated measure. Means separation was conducted using Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$).

Soil Seedbank Data Collection

Before spring tillage, 16 soil cores were randomly sampled from each plot to assess weed seedbank composition. Cores measured 5.7 cm in diameter and were collected to a depth of 7.6 cm,

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Table 1. Herbicide strategies used in both corn and soybean years.[a]

Herbicide strategy	Crop	Timing	Herbicide	Rate	WSSA SOA group no.[b]	Trade name[c]	Manufacturer
				—g ha^{-1}—			
Diversified glyphosate	Corn	PRE	Atrazine	1,100	5	Lexar® EZ	Syngenta
			S-metolachlor	1,100	15		
			Mesotrione	141	27		
		POST	Atrazine	1,120	5	AAtrex®	Syngenta
			Glyphosate	1,100	9	Roundup PowerMax®	Monsanto
			Topramazone	12	27	Impact®	AMVAC
	Soybean	PRE	Chlorimuron	18	2	Fierce® XLT	Valent
			Flumioxazin	69	14		
			Pyroxasulfone	87	15		
		POST	Glyphosate	1,426	9	Flexstar® GT	Syngenta
			Fomesafen	353	14		
Dicamba reliant	Corn	PRE	Atrazine	2,200	5	AAtrex®	Syngenta
		POST	Dicamba	560	4	XtendiMax®	Monsanto
			Glyphosate	1,100	9	Roundup PowerMax®	Monsanto
	Soybean	Early POST	Dicamba	560	4	XtendiMax®	Monsanto
			Glyphosate	1,120	9	Roundup PowerMax®	Monsanto
		Late POST	Dicamba	560	4	XtendiMax®	Monsanto
			Glyphosate	1,120	9	Roundup PowerMax®	Monsanto
Diversified dicamba	Corn	PRE	Atrazine	1,100	5	Lexar® EZ	Syngenta
			S-metolachlor	1,100	15		
			Mesotrione	141	27		
		POST	Dicamba	560	4	XtendiMax®	Monsanto
			Glyphosate	1,100	9	Roundup PowerMax®	Monsanto
	Soybean	PRE	Chlorimuron	18	2	Fierce® XLT	Valent
			Flumioxazin	69	14		
			Pyroxasulfone	87	15		
		POST	Dicamba	560	4	XtendiMax®	Monsanto
			Glyphosate	1,120	9	Roundup PowerMax®	Monsanto
Fully diversified	Corn	PRE	Atrazine	1,100	5	Lexar® EZ	Syngenta
			S-metolachlor	1,100	15		
			Mesotrione	141	27		
		POST	Atrazine	1,120	5	AAtrex®	Syngenta
			Glufosinate	450	10	Liberty®	BASF
			Topramazone	12	27	Impact®	AMVAC
	Soybean	PRE	Chlorimuron	13	2	Valor® XLT	Valent
			Flumioxazin	38	14		
		POST	Dicamba	560	4	XtendiMax®	Monsanto
			Glyphosate	1,120	9	Roundup PowerMax®	Monsanto
			Pyroxasulfone	180	15	Zidua®	BASF

[a]Table adapted from Legleiter (2017: 156–157).
[b]SOA, site of action.
[c]Before 2018 Clarity® was used in place of XtendiMax®.

Table 2. Herbicide application dates from 2013 to 2019.

Crop	Application	2013	2014	2015	2016	2017	2018	2019
Corn	PRE	May 9		May 2		April 28		May 21
	POST	June 7		June 2		June 8		June 14
Soybean	PRE		May 27		May 8		May 10	
	Early POST		June 26		June 1		June 7	
	POST		July 10		June 13		June 18	
	Late POST		July 21		June 25		July 18	
Late POST in fully diversified (2018 only)							July 3	

Table 3. ANOVA table for the influence of herbicide strategy, year, and the interaction of the two on in-field, mid-June, and soil seedbank total, dicot, and monocot density, and species richness from 2013 to 2019.

		In field: mid-June			Soil seedbank		
	Factors and interactions	Total weed species	Dicot weed species	Monocot weed species	Total weed species	Dicot weed species	Monocot weed species
		P					
Density	Herbicide strategy	<0.0001	<0.0001	<0.0001	<0.0001	0.2608	<0.0001
	Year	0.0004	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
	Herbicide strategy × year	<0.0001	<0.0001	<0.0001	0.0002	0.997	<0.0001
Species richness	Herbicide strategy	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Year	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Herbicide strategy × year	<0.0001	<0.0001	0.002	0.4649	0.842	0.0001

resulting in approximately 3,000 cm^3 of soil from each plot. Pareja et al. (1985) determined that 85% of all seeds in a reduced-tillage system, and 28% in a conventional-tillage system were in the top 5 cm of soil. Cores were homogenized and placed into 25 by 50 cm soil flats in a Purdue University greenhouse in West Lafayette, IN, where the seeds were allowed to germinate for 8 wk. Greenhouse conditions were established as a 16-h photoperiod with 600-W high-pressure sodium lights, with a temperature of approximately 26 C.

Weed density and species were recorded every 2 wk, and weeds were removed by hand after each recording date. Following data collection at the 4th week, the soil was mixed thoroughly to promote germination of additional seeds remaining in flats. After the 8-wk period was complete, the soil was discarded. Weed densities and species richness are presented per 3,000 cm^3. Other studies have presented soil seedbank densities in terms of area (e.g., m^{-2}; Carter and Ivany 2006; Conn et al. 1984; Menalled et al. 2001; Moonen and Barberi 2004). Seed density on a volume basis (cm^3) has also previously been used to compare spatial analysis methods within soil seedbanks (Bigwood and Inouye 1988). The data were analyzed all together, referred to as total and separated into monocots and dicots. All data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS v. 9.4, with year serving as a repeated measure, and means were separated using Tukey's HSD test ($\alpha = 0.05$).

Results and Discussion

Early-Summer POST Application Weed Densities and Species Richness

Total, monocot, and dicot weed densities and species richness were all influenced by an interaction between herbicide strategy and year (Table 3). Total weed density was highest in the dicamba-reliant treatments (Figure 1), and generally higher in the soybean years than in the corn years. Monocot densities increased over time in the dicamba-reliant treatment, but remained constant in the other three treatments. Dicot densities in the dicamba-reliant treatment declined over time, but like monocot densities, tended to be higher in soybean years than in corn years. By 2019, monocots accounted for more than 90% of the total weed density.

The dicamba-reliant treatment had a higher dicot species richness compared with all other treatments in years that soybeans were grown (Figure 2). This is possibly due to the lack of one dominant weed species, which allowed other dicot species to find a niche that is usually inhabited by a dominant weed species such as *A. trifida* or *Amaranthus* spp. Total, dicot, and monocot species richness were always higher in soybean years in the dicamba-reliant strategy (Figure 2). The increased species richness in soybean years is likely either due to the lack of atrazine used, or soybeans being a less competitive crop compared with corn for some weed species (Knake and Slife 1962; Moolani et al. 1963).

The research presented in this study is the first to date to evaluate weed community shifts in dicamba-resistant soybeans rotated with corn and showed that species richness will increase if dicamba is used with only glyphosate, and atrazine in corn. Shergill et al. (2017) evaluated weed shifts in dicamba-resistant continuous soybean, but did not evaluate shifts in dicamba-resistant soybeans rotated with corn. Species richness was highest in the dicamba-reliant strategy, resulting in three more species at the end of the trial compared with all other herbicide strategies (data not shown).

Using six or more SOAs with residual herbicides in the fully diversified strategy in both corn and soybean years resulted in a 98% decrease in density compared with using three SOAs with a residual only in corn years. Using six or more SOAs reduced species richness and reduced weed densities compared with a

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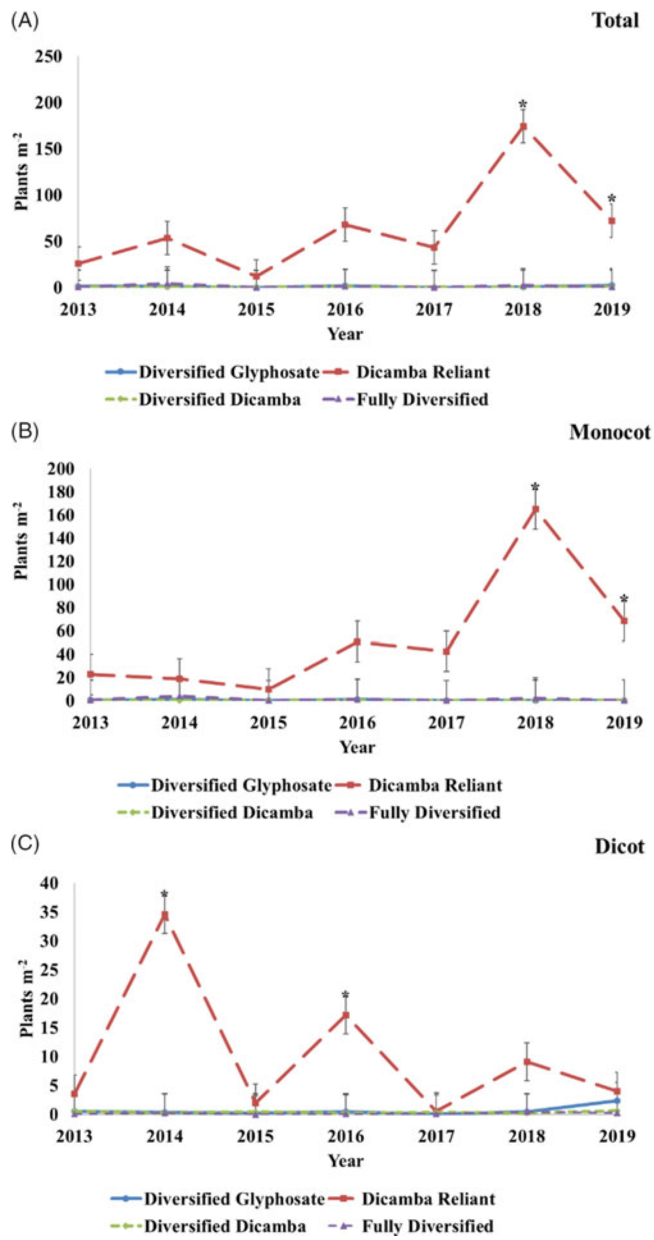

Figure 1. (A) Total, (B) monocot, and (C) dicot in-field densities at the Throckmorton Purdue Agricultural Center (8343 US-231, Lafayette, IN) in mid-June. Standard error bars shown. Asterisks represent differences in mean separation according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$) within year as influenced by an interaction between year and herbicide strategy at early-summer evaluations. Corn was grown in 2013, 2015, 2017, and 2019, and soybeans were grown in 2014, 2016, and 2018.

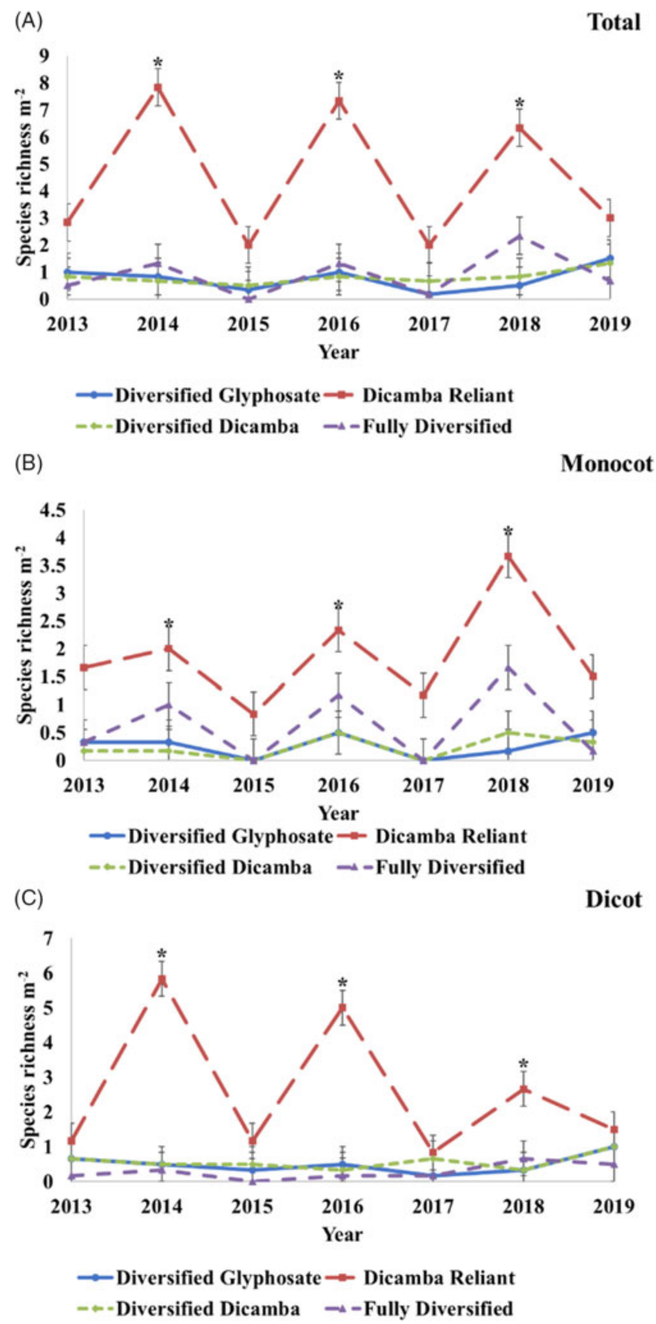

Figure 2. (A) Total, (B) monocot, and (C) dicot in-field species richness at the Throckmorton Purdue Agricultural Center (8343 US-231, Lafayette, IN) in mid-June. Standard error bars shown. Asterisks represent differences in mean separation according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$) within year as influenced by an interaction between year and herbicide strategy at early-summer evaluations. Corn was grown in 2013, 2015, 2017, and 2019, and soybeans were grown in 2014, 2016, and 2018.

herbicide strategy that only implemented dicamba and glyphosate in soybean years with the addition of atrazine in corn years. In other published research, weed populations were reduced by 50% in continuous corn when more mechanisms of action were included (Wilson et al. 2011). The addition of corn in rotation with dicamba-resistant soybeans will have large implications on weed communities due to the broad spectrum of herbicides used in corn that are not used in soybeans, such as atrazine and 4-hydroxyphenylpyruvate dioxygenase herbicides (e.g., mesotrione, tembotrione, and topramezone).

Soil Seedbank Weed Densities and Species Richness

Total and monocot weed densities within the soil seedbank were influenced by a year by herbicide strategy interaction, while dicot weed densities were only influenced by year (Table 2). Total and monocot densities showed a gradual decline from 2013 to 2015, but were highest in the dicamba-reliant strategy in 2018 and 2019 (Figure 3). The dicamba-reliant strategy had greater than 43% higher total weed densities than all other treatments due to

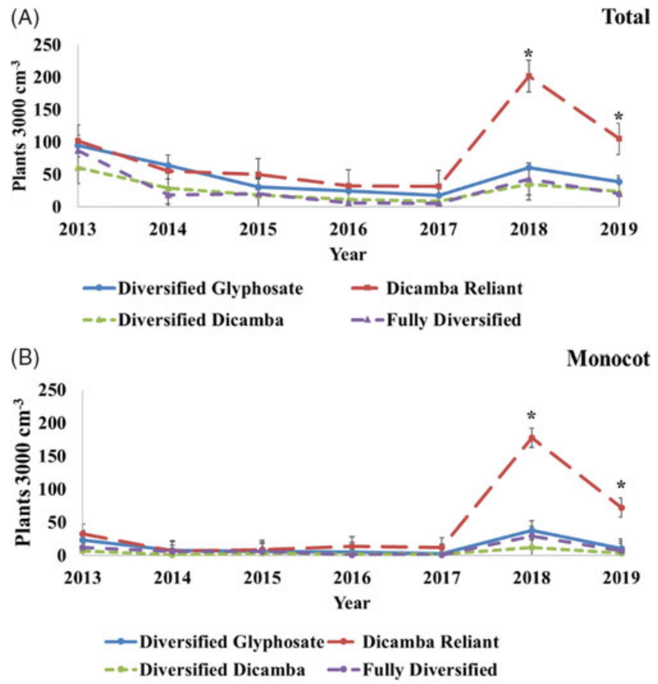

Figure 3. (A) Total and (B) monocot soil seedbank densities from the Throckmorton Purdue Agricultural Center (8343 US-231, Lafayette, IN). Standard error bars shown. Asterisks represent differences in mean separation according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$) within year as influenced by an interaction between year and herbicide strategy. Corn was grown in 2013, 2015, 2017, and 2019, and soybeans were grown 2014, 2016, and 2018.

having a higher monocot density that was at least 71% higher than in the other treatments. All herbicide strategies had similar dicot densities. A less diversified herbicide strategy with only three SOAs and no residual herbicides in soybean years over the course of 7 yr has resulted in those plots having higher total weed densities, composed primarily of monocots.

Only monocot weed species richness was influenced by a herbicide strategy by year interaction, while total and dicot weed species richness were influenced by each factor individually (Table 3). Species richness decreased as more SOAs were implemented into herbicide programs, and the fully diversified strategy resulted in the lowest dicot weed species richness, supporting the in-field observations (Table 4). The soil seedbank assessments reflects what occurred within early-summer evaluations, as the dicamba-reliant treatment had higher total weed density compared with other treatments and was primarily composed of monocots.

Crop Yield

Differences in crop yield were not observed in this research. The results from this experiment suggest that over a 7-yr period negative impacts from weed shifts on yield are not likely.

This research is the first published report to evaluate weed community response to varying levels of dicamba selection pressure in dicamba-resistant soybean rotated with corn. Soil seedbank analysis supported the observations from early-summer evaluations. Both the total and monocot weed densities in the dicamba-reliant treatment had at least 43% more weeds than the next highest treatment. The total weed densities taken in early summer were 84% monocots, while the densities within the soil seedbank were only 56% monocots. Wilson et al. (2007) also reported that in-field densities of *B. scoparia* in a 6-yr study were supported by soil seedbank

Table 4. Influence of herbicide strategy on total and dicot weed species richness in the soil seedbank averaged over 2013 to 2019.

Herbicide strategy	Species richness[a]	
	Total weed species	Dicot weed species
	——3,000 cm^{-3}——	
Diversified glyphosate	4.9 ab	3.4 a
Dicamba reliant	5.4 a	3.2 a
Diversified dicamba	4.1 bc	3.2 a
Fully diversified	3.4 c	2.2 b

[a]Means followed by the same letter with columns are not different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$).

analysis, as both decreased due to varying herbicides strategies. We demonstrated that utilizing six or more SOAs and residual herbicides in both corn and soybean years reduced weed densities compared with three SOAs and using a residual herbicide only in corn years. Shergill et al. (2017) found similar results, as residual herbicides applied before POST glyphosate applications resulted in a greater than 50% reduction in weed densities and a 250% increase in yield compared with glyphosate alone. However, reduction in weed densities and increase in yield were not observed in the first year, but after the 4th year.

Species richness within the dicamba-reliant treatment was higher for total, dicot, and monocots by 3.5, 2, and 1.3 species, respectively, compared with all other herbicide strategies at early-summer evaluations. The other three treatments did not differ from one another. A similar result occurred within the soil seedbank, as the dicamba-reliant treatment had 0.7 more species than the next highest treatment. A shift into more diverse weed species occurred due to fewer SOAs being implemented in both corn and soybean years, but more importantly due to the lack of a residual herbicide in years when soybean was grown. Shifts in weed seedbanks were expected due to the changes in herbicide management practices, as differences in arable weed seedbanks have been observed due to differences in production practices in both organic and conventional cropping systems (Rotchés-Ribalta et al. 2017). Ovejero et al. (2013) and Tharp and Kells (2002) have shown that the use of residual herbicides increases overall weed control. Jhala et al. (2017) reported that soil-applied residual herbicides followed by residual POST applications provided 82% control of common waterhemp [*Amaranthus rudis* (Moq.) Sauer], while strategies without a PRE herbicide reported 45% control at harvest. Using sequential applications of soil-residual herbicides also reduced weed densities and weed species richness in the present study.

We demonstrated that farm operators need to utilize both multiple herbicide SOAs and sequential applications of residual herbicides to decrease the densities and species richness of weed communities in corn rotated with dicamba-resistant soybeans, which has not been previously evaluated.

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