#### ARTICLE

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# Dose response of yellow and white popcorn hybrids to glyphosate, a premix of 2,4-D choline and glyphosate, or dicamba

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#### **Abstract**

Popcorn (Zea mays L. var. everta) is difficult to distinguish from field corn (Zea mays L. var. indentata) and is produced adjacent or in close proximity to field corn and soybean [Glycine max (L.) Merr.]. This creates a potential for misapplication or drift of herbicides used in nearby corn and soybean. Field experiments were conducted near Clay Center, Nebraska in 2017 and 2018 to determine the effect of glyphosate, 2,4-D choline/glyphosate (premix), or dicamba on the growth and yield of popcorn. Treatments included nontreated control and four rates of glyphosate, 2,4-D choline/glyphosate, or dicamba applied post-emergence (POST) at five- or eight-leaf (V5 and V8, respectively) popcorn growth stages to white and yellow popcorn hybrids. A three-parameter log-logistic model was fitted to treatment combination with varying herbicide rates. Glyphosate and 2,4-D choline/glyphosate applied at V5 had greater injury, biomass reduction, height reduction, and yield loss than at V8. The two hybrids responded similarly to herbicides at most measurements. At the V8 application, 2,4-D choline/glyphosate resulted in greater injury in the white hybrid; however, no hybrid differences in glyphosate sensitivity were observed at the V8 growth stage. Glyphosate and 2,4-D choline/glyphosate at 0.25× rates resulted in complete plant death in both hybrids, whereas the highest dicamba dose (2x) caused 11% injury and no biomass reduction, plant height reduction, or yield loss. These results demonstrate the sensitivity of popcorn to glyphosate, 2,4-D choline/glyphosate, or dicamba and can be of immediate educational use and practical implementation for popcorn producers, herbicide applicators, and agronomists.

# 1 | INTRODUCTION

Popcorn (*Zea mays* L. var. everta) is an important specialty crop for many traditional field corn (*Zea mays* L. var. indentata) and soybean [*Glycine max* (L.) Merr.] producers

5% response occurs;  $ED_{50}$ , herbicide rate where 50% response occurs between lower and upper limit and inflection point; ME, modeling efficiency; POST, post-emergence; PRE, pre-emergence

**Abbreviations:** DAT, days after treatment; ED<sub>5</sub>, herbicide rate where

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in the Midwestern United States, generally contributing to greater farm income (Edleman, 2004; 2006). Popcorn is grown on nearly 90,000 ha in the United States (USDA NASS, 2019) with the major popcorn-producing states being Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Nebraska, and Ohio with Nebraska leading at 45% of the total production (USDA NASS, 2019). Popcorn is normally produced under contract with a private company or manufacturer that specifies the hybrids and areas to be planted (D'Croz-Mason & Waldren, 1978; Ziegler, 2001).

Popcorn is generally more susceptible to herbicide injury and less competitive with weeds than field corn (Pike et al., 2002; Ziegler, 2001). Popcorn emerges slower, produces narrower and more upright leaves, and tends to have shorter and thinner stalks than field corn (Ziegler, 2001). Herbicide labels often exclude white popcorn as it is thought to be more sensitive to herbicides than yellow popcorn (Loux et al., 2017). Exclusionary labels include premixes of atrazine/mesotrione/S-metolachlor (Syngenta Crop Protection, 2009; Syngenta Crop Protection, 2012), acetochlor/mesotrione/clopyralid (Corteva Agriscience, 2017b), atrazine/bicyclopyrone/mesotrione/Sand metolachlor (Syngenta Crop Protection, 2017). Despite the general perception that white popcorn hybrids are less tolerant to herbicides, evidence has not been published. Transgenic, herbicide-resistant hybrids of popcorn are not commercially available; therefore, popcorn is not resistant to commonly used post-emergence (POST) herbicides such as glyphosate or glufosinate in much of the field corn grown in the United States (Fernandez-Cornejo, Wechsler, Livingston, & Mitchell, 2014; Ziegler, 2001).

A statewide survey reported glyphosate as the most used POST herbicide in glyphosate-resistant corn and soybean in Nebraska (Sarangi and Jhala, 2018a). According to Kniss (2017), 90% of the field corn area was treated with glyphosate in 2014 and 140% (multiple applications) of soybean area was treated with glyphosate in 2015 in the United States. This accounts for 26 and 43% of all herbicide treatments in corn and soybean, respectively. Weed Science Society of America's Group 4 site of action herbicides (synthetic auxins; Hernicide Resistance Action Committee [HRAC] Group O), are applied on 36% of corn area on average from 1990 to 2014 (Kniss, 2017). Dicamba use has increased in recent years with rapid evolution of glyphosate-resistant weed biotypes and the commercialization of dicamba-resistant soybean. In Nebraska, about 19% of the 2.3 million ha of soybean planted in 2017 were dicamba-resistant, of which an estimated 80% were treated with dicamba (Werle et al., 2018). Bayer Crop Science estimated 16.2 million ha planted to dicambaresistant soybean in 2018 or around half of the soybean production area in the United States (Werle et al., 2018). The adoption of dicamba-resistant soybean increased to

#### **Core Ideas**

- Popcorn producers operate in landscapes dominated by herbicide-resistant corn and soybean.
- Yield loss was >50% with one-sixteenth of the labeled rates of glyphosate and 2,4-D choline/ glyphosate.
- Yellow and white popcorn hybrids were less sensitive to glyphosate and 2,4-D choline/ glyphosate at V8 stage than V5 stage.
- The response of the yellow and white popcorn hybrids to glyphosate and 2,4-D choline/ glyphosate was similar for most measurements.
- Dicamba at two times the labeled rate resulted in 11% popcorn injury but no biomass or yield reduction.

about 50% of total soybean planted in Nebraska for 2018 (Jhala, 2018). In 2018, 94% of the United States and 96% of Nebraska soybean planted had an herbicide-resistant trait (USDA NASS, 2019). The possible herbicide-resistant traits in soybean include glyphosate, glyphosate plus dicamba (combined traits), glufosinate, or 2,4-D plus glyphosate plus glufosinate (combined traits; Nandula, 2019). Herbicide-resistant field corn represents 80% of the field corn in the United States and 84% of Nebraska field corn in 2018 (USDA NASS, 2019). Corn resistant to 2,4-D choline plus glyphosate plus glufosinate was commercialized in 2018 (Nandula, 2019). That outcome will inevitably increase the use of 2,4-D choline particularly for control of glyphosate-resistant common waterhemp [Amaranthus tuberculatus (Moq.) J. D. Sauer] and Palmer amaranth (Amaranthus palmeri S. Watson) in Nebraska.

Popcorn is grown in proximity primarily to corn, soybean, and wheat (Triticum aestivum L.) in the Midwest. The current USDA pest management strategic plan for popcorn has prioritized regulation, research, and education for enhancing popcorn production and production efficiency (Pike et al., 2002). The strategic plan outlined the importance of educating custom applicators about the lack of herbicide-resistant traits in popcorn compared to field corn, and the necessity of checking herbicide labels for special instructions or reduced rates regarding popcorn when herbicides are labeled for both crops (Pike et al., 2002). One regulatory priority in the strategic plan is the critical issue of hybrid sensitivity to new herbicides as they are being registered for use in corn and soybean with the potential of drift, tank contamination, or carryover injury to popcorn (Pike et al., 2002).

Conventional field corn response to glyphosate has been documented in several studies (Brown et al., 2009; Buehring, Massey, & Reynolds, 2007; Ellis, Griffin, & Jones, 2002; Ellis, Griffin, Linscombe, & Webster, 2003; Reddy, Bellaloui, & Zablotowicz, 2010). Additionally, Banks and Schroeder (2002) reported the response of sweet corn to simulated drift rates of glyphosate. Growth stage has been reported to influence herbicide efficacy in field corn in simulated drift studies (Ellis et al., 2003; Reddy et al., 2010). Popcorn production fields in Nebraska are commonly grown in rotation with soybean and field corn and thus are in close proximity to corn and soybean production fields. Herbicides applied POST in corn and soybean, such as glyphosate, 2,4-D choline, or dicamba, may result in off-target movement and popcorn injury. Misapplication, drift, and tank contamination risk of glyphosate, 2,4-D choline/glyphosate (premix), or dicamba to popcorn fields has not been assessed. The objective of this research was to determine the effect of simulated drift rates of glyphosate, 2,4-D choline/glyphosate (premix), or dicamba on the injury, above ground biomass, height reduction, and yield loss of yellow and white popcorn hybrids at two growth stages.

#### 2 | MATERIALS AND METHODS

# 2.1 | Site description

Field experiments were conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE (40.5752°N, 98.1428°W and 552 m above mean sea level) in 2017 and 2018. The soil texture at the experimental site was Hastings silt loam (montmorillonitic, mesic, Pachic Argiustolls; with particle size distribution of 17% sand, 58% silt, and 25% clay) with a pH of 6.5, and 2.5–3% organic matter. The experimental site was disked before planting with a tandem disk at a depth of 10 cm and fertilized with 202 kg ha<sup>-1</sup> of nitrogen in the form of anhydrous ammonia (82–0–0) and was irrigated using a linear-move irrigation system.

# 2.2 | Experimental design and treatments

The treatments were a 2×2×3×5 factorial design comprising of two hybrids, two growth stages, and three herbicides with five herbicide rates. The study was laid out in a randomized complete block design and included three replications each year. Popcorn hybrids consisted of a commonly grown white (VWP111; Conagra Brands, Chicago, IL) and yellow (VYP315; Conagra Brands) popcorn hybrids. Herbicides included a nontreated control in each hybrid as the common 0× rate, glyphosate at

four rates (0.25×, 0.125×, 0.063×, and 0.031×), 2,4-D choline/glyphosate (premix) at four rates (0.25×, 0.125×, 0.5×, and 0.25×) applied POST at V5 or V8 popcorn growth stages. The glyphosate (479.3 g as  $L^{-1}$ ) labeled rate (1×) is 1.680 g ae ha<sup>-1</sup> for glyphosate-resistant corn and soybean (Durango, Corteva, Indianapolis, IN). The premix of 2,4-D choline/glyphosate (191.7 g ae L<sup>-1</sup> of 2,4-D choline [48.5%]; 203.7 g ae  $L^{-1}$  of glyphosate [51.5%]) labeled rate (1X) is 2,200 g ae ha<sup>-1</sup> for 2,4-D/glyphosate resistant corn and soybean (Enlist DUO, Corteva, Indianapolis, IN). The dicamba (350 g ae  $L^{-1}$ ) labeled rate (1x) is 560 g ae ha<sup>-1</sup> for dicamba-resistant soybean (XtendiMax, Bayer CropScience 2018, Research Triangle Park, NC). The rates of glyphosate and 2,4-D choline/glyphosate are similar to the glyphosate rates tested in the literature on sweet and field corn (Banks & Schroeder, 2002; Buehring et al., 2007). Dicamba is labeled in popcorn; therefore, a maximum of 2× of the labeled rate was selected as a worst-case scenario.

Plot dimensions were 9-m long by 3-m wide. On 27 April 2017 and 26 April 2018, popcorn hybrids were planted in rows spaced 76 cm apart at 4-cm depth with a planting density of 89,000 seeds ha<sup>-1</sup>. Ammonium polyphosphate (APP; 10-34-0) was applied in furrow as starter fertilizer at 6 kg ha<sup>-1</sup> during planting. A premix of atrazine/S-metolachlor (Bicep II Magnum, Syngenta Crop Protection, Greensboro, NC) was applied pre-emergence (PRE) at 2,470 g ai ha<sup>-1</sup> for early season weed control on 27 April 2017 and 2 May 2018. The premix of atrazine/S-metolachlor consists of 33% atrazine and 26.1% S-metolachlor active. Post-emergence herbicide treatments were applied when popcorn reached V5 or V8 growth stages, 14 and 29 June in 2017 and 31 May and 18 June in 2018, using a handheld CO<sub>2</sub>-pressurized backpack sprayer equipped with four AIXR 110015 flat-fan nozzles (TeeJet Technologies, Spraying Systems, Wheaton, IL) spaced 51 cm apart and calibrated to deliver 140 L ha<sup>-1</sup> at 276 kPa at a constant speed of 4.8 km  $h^{-1}$ .

# 2.3 | Data collection

Air temperature and rainfall data were obtained from the High Plains Regional Climate Center automated weather station that was located 350 m from the experimental field. Popcorn injury was assessed on a scale of 0 to 100%, with 0% representing no injury and 100% representing plant death at 21 d after V5 application and 21 d after V8 application. Popcorn plant height was measured from 6 plants plot<sup>-1</sup> at 21 d after V5 and V8 applications by measuring from the soil surface to the arch of the tallest collared leaf of each plant. Aboveground popcorn biomass was collected from four sequential plants in the middle two rows from

each plot at 70 d after V8 application. Plants were cut near the soil surface, put in paper bags, and dried in an oven at 50 °C for 10 d to constant weight, and dry biomass weight was recorded. Percent biomass reduction and percent height reduction compared with the nontreated control was calculated using the equation (Wortman, 2014):

$$Y = [(C - B)/C] 100 (1)$$

where, Y represents the percent biomass reduction or height reduction compared to the nontreated control plot in the corresponding replication block, C represents the biomass or height from the nontreated control plot, and B represents the biomass or height of the treatment plot. Popcorn was harvested with a plot combine from the middle two rows to avoid edge effect and the yields were adjusted to 14% grain moisture content. Relative yield loss was calculated as:

$$YL = 100 (1 - P/C)$$
 (2)

where, YL is the yield loss relative to the nontreated control, *P* is the plot yield, and *C* is the yield of the nontreated control.

# 2.4 | Statistical analysis

R (R Core Team, 2019) base packages and the *drc: Analysis of Dose-Response Curves* package (Ritz, Baty, Streibig, & Gerhard, 2015) were utilized for data analysis. Injury, height reduction, biomass reduction, and relative yield loss data were analyzed using the three-parameter log-logistic model (Knezevic, Streibig, & Ritz, 2007):

$$Y = D/\{1 + \exp[B(\log X - \log E)]\}$$
 (3)

where, Y is popcorn injury, biomass reduction, height reduction, or yield loss, D is the upper limit (maximum effect; not allowed to exceed 100%), E is the herbicide rate where 50% response between lower and upper limit occurs; inflection point (ED<sub>50</sub>), and B is the slope of the line at the inflection point. Models fit with separate curves for fixed effects (hybrid and growth stage) or random effects (years) were subjected to F-tests separated at  $\alpha = .05$  to test for significance of effects and compared to the overall model not distinguishing between years or hybrid and growth stage combination (single curve). Data were pooled over years if the random effect of year was not significant. When the treatment effect was significant, each treatment (hybrids and growth stage combinations) was fitted to the model separately. Model parameters, B, D, and E, and the herbicide rate causing 5% response (ED<sub>5</sub>) were statistically compared between hybrids and growth stages using T-tests separated at  $\alpha = .05$  utilizing the *compParm* function in R. A Pearson's correlation analysis was conducted between popcorn yield loss and popcorn injury, biomass reduction, and height reduction (Deeds, Al-Khatib, Peterson, & Stahlman, 2006).

# 2.5 | Model goodness of fit

Root mean squared error (RMSE) and modeling efficiency (ME) were calculated to evaluate goodness of fit for popcorn injury, biomass reduction, height reduction, and yield loss models. The RMSE was calculated with equation (Barnes, Jhala, Knezevic, Sikkema, & Lindquist, 2018; Roman, Murphy, & Swanton, 2000; Sarangi and Jhala, 2018b):

RMSE = 
$$[1/n \sum_{i=1}^{n} (Pi - Oi)^2]^{1/2}$$
 (4)

where, *Pi* and *Oi* are the predicted and observed values, respectively, and *n* is the total number of comparisons. The smaller the RMSE, the closer the model predicted values to the observed values. The ME was calculated using following equation (Barnes et al., 2017; Mayer & Butler, 1993):

$$ME = 1 - \left[ \sum_{i=1}^{n} (Oi - Pi)^{2} / \sum_{i=1}^{n} (Oi - Oi)^{2} \right]$$
 (5)

where, O is the mean observed value and all other parameters are the same as equation 4. ME differs from coefficient of determination  $(r^2)$  only in not having a lower limit. ME values closest to 1 indicate the most accurate (perfect) predictions (Sarangi, Irmak, Lindquist, Knezevic, & Jhala, 2015).

#### 3 | RESULTS AND DISCUSSION

The research site received near average precipitation and supplemental irrigation via a linear pivot. Total seasonal water from April through September was 64 cm in 2017 and 65 cm in 2018. Temperatures at the research site were near normal throughout the two growing seasons. The fits of individual years were not significantly different compared to the combined model for any response variable (injury, height reduction, biomass reduction, and yield loss); therefore, data were pooled across years (Table 1). When curves were fitted to each hybrid and growth stage combination the fits differed from that of a single curve, with both hybrids and growth stages combined,

TABLE 1 P-values of F-test ( $\alpha$  < .05) between three-parameter log-logistic model with year or hybrid and growth stage (combined across years because year was non-significant) effect in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018

Herbicide	Response	Treatment	Year
Glyphosate	injury	<.001	.137
	$biomass\ reduction$	<.001	.244
	height reduction	<.001	.710
	yield loss	<.001	.288
2,4-D choline/glyphosate	injury	<.001	.772
	$biomass\ reduction$	<.001	.201
	height reduction	<.001	.570
	yield loss	.004	.105
Dicamba	injury	1	.614
	biomass reduction	NA <sup>a</sup>	NA
	height reduction	NA	NA
	yield loss	NA	NA

<sup>&</sup>lt;sup>a</sup>NA, not applicable.

for glyphosate and 2,4-D choline/glyphosate but not for dicamba visual estimates of crop injury (Table 1). Dicamba estimates of visual injury were pooled across hybrids and growth stages and a single curve was fitted.

# 3.1 | Growth stage

Popcorn injury response to glyphosate was greatest at the V5 growth stage (Figure 1a). The ED<sub>50</sub> values at the V5 growth stage averaged 23 g ae ha<sup>-1</sup> compared with 73 g ae ha<sup>-1</sup> at the V8 growth stage (Table 2). The same trend was observed in the ED<sub>5</sub> values with glyphosate applied at the V5 growth stage averaging 5 g ae ha<sup>-1</sup> compared with 46 g ae ha-1 at the V8 growth stage. A greater slope resulted from the V5 than for the V8 growth stage application. The upper limit reached or exceeded 100% injury for both growth stages although was not allowed to exceed 100%. With conventional field corn, Reddy et al. (2010) reported 45 and 55% injury 21 d after treatment (DAT) when glyphosate at 105 g ae ha<sup>-1</sup> was applied at V2-V4 and V6-V8 growth stages, respectively. Results of dose response in this study predicted 105 g ae ha<sup>-1</sup> of glyphosate to result in 71-78% and 56-60% injury across the two hybrids when applied at the V5 and V8 popcorn growth stages, respectively. This suggests that popcorn in this research is more sensitive to glyphosate than conventional field corn reported by Reddy et al. (2010). Ellis et al. (2003) reported 140 g ae ha<sup>-1</sup> of glyphosate resulted in 64-78% injury 7 DAT when applied at the V6 conventional field corn growth stage and 0–40% injury at 7 DAT when applied at the V9 growth stage. Glyphosate at 140 g ae ha<sup>-1</sup> resulted in popcorn injury of 75–82% and 73–76% when applied at the V5 and V8 popcorn growth stage, respectively, which is lower than that reported in the literature for conventional field corn. In general, glyphosate application at the V5 growth stage resulted in greater biomass reduction than the V8 growth stage, which may indicate greater sensitivity of popcorn to herbicide at V5 stage (Figure 1c). The ED<sub>50</sub> and ED<sub>5</sub> were lower for the V5 growth stage than the V8 growth stage (Table 3). The slope and upper limit were similar between both growth stages.

Glyphosate applied at the V5 growth stage resulted in greater height reductions than application at the V8. For example, ED<sub>50</sub> for glyphosate applied at the V5 stage averaged 41 g ae ha<sup>-1</sup> for the two hybrids compared with 82 g ae ha<sup>-1</sup> when applied at the V8 application. Similarly, a greater amount of glyphosate was needed to reduce popcorn height by 5% (ED<sub>5</sub>) at the V8 growth stage (Figure 1e). The maximum popcorn height reduction (upper limit) following glyphosate application was greater for the V5 application (91%) than the V8 application (75%) for the white hybrid (Table 4). This was not the case for the yellow hybrid with maximum height reduction not statistically differing between V5 and V8 model fits. The slopes were similar between hybrids and growth stages. Ellis et al. (2003) reported 10-87% height reduction from 140 g ae ha<sup>-1</sup> of glyphosate application at 7 DAT at the V6 conventional corn growth stage and 4-32% reduction at 7 DAT at the V9 growth stage.

Glyphosate applied at the V5 growth stage resulted in greater yield loss than the V8 application (Figure 1g). For instance, the  $\rm ED_{50}$  averaged 16 g ae ha<sup>-1</sup> for the two hybrids when applied at the V5 stage and 64 g ae ha<sup>-1</sup> at the V8 growth stage (Table 5). The  $\rm ED_5$  followed a similar trend as the  $\rm ED_{50}$  shifting from 1 g ae ha<sup>-1</sup> at the V5 stage to 13 g ae ha<sup>-1</sup> at the V8 growth stage. The slope and upper limit did not vary between growth stages, suggesting that the range of effects were similar between growth stages and that the rate of change in response of herbicide rate was similar. Ellis et al. (2003) reported 78 and 33% yield loss with glyphosate at 140 g ae ha<sup>-1</sup> applied at the V6 and V9 growth stages, respectively, in conventional corn.

Similar to glyphosate alone, 2,4-D choline/glyphosate applied at the V5 stage resulted in more injury than when applied at the V8 growth stage (Figure 1b). For example, the white popcorn hybrid at the V5 growth stage resulted in  $ED_{50}$  of 35 g ae ha<sup>-1</sup> compared with 77 g ae ha<sup>-1</sup> at the V8 stage (Table 2). Similarly, the yellow popcorn hybrid at the V5 growth stage resulted in  $ED_{50}$  of 72 g ae ha<sup>-1</sup> compared with 98 g ae ha<sup>-1</sup> at the V8 stage. The  $ED_5$  was similar for the V5 and V8 growth stages in the yellow popcorn hybrid (23 and 31 g ae ha<sup>-1</sup>); however, the  $ED_5$  in the white

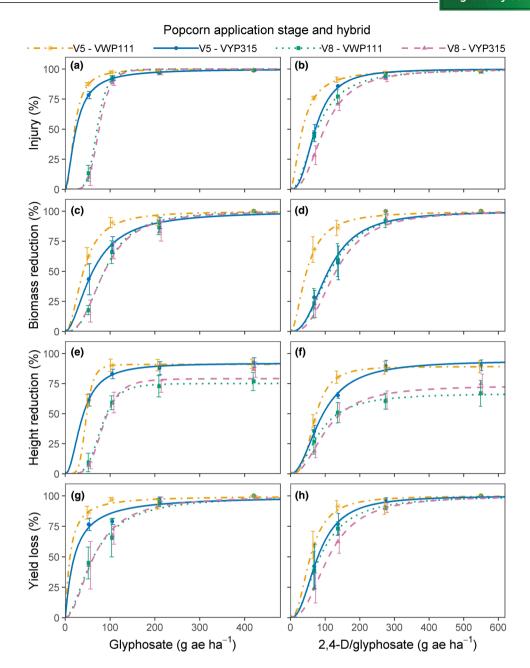


FIGURE 1 Dose-response of two popcorn [Zea mays (L.) var. everta] hybrids (VWP111, white kernel color; and VYP315, yellow kernel color; Conagra Brands) at two growth stages (V5, five-leaf stage; V8, eight-leaf stage) to glyphosate and 2,4-D choline/glyphosate based on (a, b) visual assessment of injury, (c, d) biomass reduction, (e, f) height reduction, and (g, h) yield loss combined across years in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, Nebraska in 2017 and 2018. Regression lines represent the fit of a three-parameter log-logistic model. Error bars represent standard error of treatment means.

popcorn hybrid was 6 and 19 g ae  $ha^{-1}$ , respectively. The slope between the V5 and V8 applications within hybrids were not significantly different, which generally results in the same dose-response shape even if the  $ED_{50}$  is shifted between hybrids or growth stages. The application of 2,4-D choline/glyphosate at the V5 growth stage resulted in a similar biomass reduction compared to the V8 growth stage in the yellow hybrid (Figure 1d). However, with the

white hybrid, greater biomass reduction occurred following the V5 application compared to the V8 application with  $\rm ED_{50}$  of 47 g ae  $\rm ha^{-1}$  compared with 114 g ae  $\rm ha^{-1}$ , respectively (Table 3). Popcorn height reduction and yield loss due to 2,4-D choline/glyphosate was similar between hybrids (Figures 2f, 2h; Tables 4, 5).

Dicamba injury ranged from 1% ( $\pm 0.5$ ) at 0.25× rate (140 g ae ha<sup>-1</sup>) to 11% ( $\pm 2.3$ ) at the 2× rate (1,120 g ae ha<sup>-1</sup>).

2962 Agronomy Journal BARNES ET AL.

TABLE 2 Parameter estimates (b, d, and e) and ED<sub>5</sub> with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [ $Zea\ mays\ (L.)\ var.\ everta$ ] hybrids (VWP111, white kernel color, Conagra brand; VYP315, yellow kernel color, Conagra brand) at two growth stages to glyphosate, 2,4-D choline/glyphosate, and dicamba based on visual observations of injury combined across years in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018

Herbicide	Stage	Hybrid	Slope	Upper limit	ED <sub>50</sub> a	$ED_5$	RMSE	ME
				% injury	g ae ha <sup>-1</sup>		% injury	
Glyphosate	V5	VWP111	$-2.1 (\pm 1.1)^{b} a^{c}$	$100 (\pm 0)$	21 (±11)a	5 (±7)a	1.6	1.0
		VYP315	$-1.7 (\pm 0.4)a$	$100 (\pm 0)$	24 (±6)a	$4(\pm 3)a$	3.8	.99
	V8	VWP111	$-6.2 (\pm 0.5)$ b	$100 (\pm 0)$	71 (±2)b	44 (±2)b	7.3	.97
		VYP315	$-6.1 (\pm 0.5)$ b	$100  (\pm 0)$	75 (±2)b	$47(\pm 2)b$	7.4	.97
2,4-D choline/glyphosate	V5	VWP111	$-1.7 (\pm 0.5)a$	$100 (\pm 0)$	35 (±9)a	6 (±4)a	2.9	.99
		VYP315	$-2.6 (\pm 0.4)a$	$100 (\pm 0)$	72 (±3)b	23 (±4)bc	7.7	.96
	V8	VWP111	$-2.1(\pm 0.3)a$	$100 (\pm 0)$	77 ( <u>±</u> 4)b	19 (±4)b	7.5	.96
		VYP315	$-2.5 (\pm 0.3)a$	$100 (\pm 0)$	98 (±4)c	$31(\pm 4)c$	9.9	.94
Dicamba	pooled	pooled	$-1.5(\pm0.4)$	$100 (\pm 0)$	4403 (±1665)	643 (±81)	5.4	.33

 $<sup>^{</sup>a}$ ED<sub>50</sub>, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED<sub>5</sub>, herbicide rate where 5% response occurs; RMSE, root mean square error; ME, modeling efficiency.

TABLE 3 Parameter estimates (*b, d,* and *e*) and ED<sub>5</sub> with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [*Zea mays* (L.) var. everta] hybrids (VWP111, white kernel color, Conagra brand; VYP315, yellow kernel color, Conagra brand) at two growth stages to glyphosate and 2,4-D choline/glyphosate based on biomass reduction combined across years in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018

Herbicide	Stage	Hybrid	Slope	Upper limit	ED <sub>50</sub> a	$ED_5$	RMSE	ME
				% BMr	g ae ha <sup>-1</sup>		% BMr	
Glyphosate	V5	VWP111	$-2.1 (\pm 0.8)^{b} a^{c}$	$100 (\pm 0)$	51 (±7)a	10 (±7)a	9.4	.94
		VYP315	$-1.8 (\pm 0.4)a$	$100 (\pm 0)$	62 (±6)b	12 (±5)ab	15.5	.84
	V8	VWP111	$-2.8 (\pm 0.5)a$	$100 (\pm 0)$	$87(\pm 6)c$	30 (±6)bc	12.3	.91
		VYP315	$-2.6 \ (\pm 0.5)a$	$100 (\pm 0)$	88 (±6)c	29 (±6)c	14.4	.87
2,4-D choline/glyphosate	V5	VWP111	$-1.9 (\pm 0.8)a$	$100 (\pm 0)$	47 (±12)a	10 (±9)a	12.5	.90
		VYP315	$-2.5 (\pm 0.5)a$	$100 (\pm 0)$	109 (±10)b	34 (±9)b	16.1	.85
	V8	VWP111	$-2.5 (\pm 0.5)a$	$100 (\pm 0)$	114 (±10)b	35 (±9)b	19.3	.80
		VYP315	$-2.7 (\pm 0.6)a$	$100 (\pm 0)$	131 (±12)b	45 (±12)b	14.8	.88

 $<sup>^{</sup>a}$ ED<sub>50</sub>, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED<sub>5</sub>, herbicide rate where 5% response occurs; ME, modeling efficiency; RMSE, root mean squared error; BMr, biomass reduction.

The injury observed from the  $2\times$  rate of dicamba consisted of brace root malformation and mid-season lodging followed by goose-necking. There was no significant difference among the four curves (hybrids or growth stage for dicamba injury); therefore, data were combined, and a single curve was obtained (Tables 1, 2; Figure 2). The labeled rate of dicamba is 560 g ae ha<sup>-1</sup>, while the ED<sub>5</sub> value for dicamba injury was 643 g ae ha<sup>-1</sup> ( $\pm$ 81). There was no popcorn biomass reduction, height reduction, or yield

loss from dicamba applications regardless of rate or growth stage. Late application of dicamba in field corn has been shown to result in injury consisting of fused brace roots, stalk bending and brittleness, or missing kernels similar to symptoms with late application of 2,4-D (Gunsolus & Curran, 1999). Dicamba (XtendiMax) used in this study is labeled in popcorn; however, it can be applied up to V5 growth stage and the label recommends verification with supplier for popcorn sensitivity (Bayer Crop Science, 2018).

<sup>&</sup>lt;sup>b</sup>Values in parentheses are standard error of parameter estimates.

 $<sup>^{</sup>c}$ Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where  $\alpha = .05$ .

<sup>&</sup>lt;sup>b</sup>Values in parentheses are standard error of parameter estimates.

 $<sup>^{</sup>c}$ Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where  $\alpha = .05$ .

TABLE 4 Parameter estimates (b, d, and e) and ED<sub>5</sub> with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [ $Zea\ mays\ (L.)\ var.\ everta$ ] hybrids (VWP111, white kernel color, Conagra brand; VYP315, yellow kernel color, Conagra brand) at two growth stages to glyphosate and 2,4-D choline/glyphosate based on height reduction combined across years in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018

Herbicide	Stage	Hybrid	Slope	Upper limit	ED <sub>50</sub> a	$ED_5$	RMSE	ME
				% HR	g ae ha <sup>-1</sup>		% HR	
Glyphosate	V5	VWP111	$-5.8 (\pm 11.6)^{b} a^{c}$	90.9 (±3.6)b	47 (±11)a	29 (±35) ab	9.4	.93
		VYP315	$-2.1(\pm 1.3)a$	92.0 (±5.7)b	38 (±11)a	10 (±10)a	9.4	.93
	V8	VWP111	$-4.7 (\pm 1.1)a$	75.1 (±3.9)a	80 (±6)b	45 (±7)ab	14.7	.83
		VYP315	−4.8 (±1.3)a	79.1 (±4.2)ab	84 (±6)b	48 (±8)b	12.0	.89
2,4-D choline/glyphosate	V5	VWP111	−3.2 (±1.4)a	89.1 (±4.8)b	69 (±6)a	29 (±11)a	10.3	.92
		VYP315	$-2.1 (\pm 0.6)a$	94.2 (±6.9)b	89 (±11)ab	23 (±9)a	8.8	.94
	V8	VWP111	−2.1 (±1.1)a	67.0 (±7.4)a	83 (±15)ab	25 (±14)a	18.5	.64
		VYP315	$-2.3 (\pm 1.0)a$	73.5 (±8.3)ab	105 (±19)b	33 (±14)a	15.1	.77

 $<sup>^{</sup>a}$ ED<sub>50</sub>, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED<sub>5</sub>, herbicide rate where 5% response occurs; RMSE, root mean squared error; ME, modeling efficiency; HR, height reduction.

TABLE 5 Parameter estimates (b, d, and e) and ED $_5$  with standard errors of the three-parameter log-logistic model, root mean squared error, and modeling efficiency used to determine the dose-response of two popcorn [ $Zea\ mays\ (L.)\ var.\ everta]$  hybrids (VWP111, white kernel color, Conagra brand; VYP315, yellow kernel color, Conagra brand) at two growth stages to glyphosate and 2,4-D choline/glyphosate based on yield loss combined across years in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018

Herbicide	Stage	Hybrid	Slope	Upper limit	ED <sub>50</sub> a	$ED_5$	RMSE	ME
				% YL	g ae ha <sup>-1</sup>		% YL	
Glyphosate	V5	VWP111	$-1.2 (\pm 1.4)^{b} a^{c}$	$100 (\pm 0)$	11 (±23)a	1 (±5)a	6.5	.97
		VYP315	$-1.1 (\pm 0.5)a$	$100 (\pm 0)$	21 (±12)ab	1 (±3)ab	6.6	.97
	V8	VWP111	$-1.8 (\pm 0.4)a$	$100 (\pm 0)$	63 (±8)bc	12 (±5)bc	19.8	.78
		VYP315	$-2.0 \ (\pm 0.4)a$	$100  (\pm 0)$	64 (±7)c	14 (±5)c	18.5	.80
2,4-D choline/glyphosate	V5	VWP111	$-2.3 (\pm 1.2)a$	$100 (\pm 0)$	57 (±11)a	16 (±13)a	14.2	.87
		VYP315	$-2.3 \ (\pm 0.6)a$	$100  (\pm 0)$	79 (±9)ab	21 (±8)a	19.9	.79
	V8	VWP111	$-2.1 (\pm 0.5)a$	$100 (\pm 0)$	86 (±10)bc	22 (±9)a	16.7	.83
VYP315	$-2.4 (\pm 0.5)a$	$100 (\pm 0)$	110 (±11)c	33 (±10)a	16.3	.84		

 $<sup>^{</sup>a}$ ED<sub>50</sub>, herbicide rate where 50% response between lower and upper limit occurs and inflection point; ED<sub>5</sub>, herbicide rate where 5% response occurs; RMSE, root mean squared error; ME, modeling efficiency; YL, yield loss.

Although information regarding popcorn's sensitivity to dicamba is not available in the published literature, sweet corn sensitivity to dicamba has been researched and has been shown to be linked to a mutation of a P450 gene (Nordby, Williams, Pataky, Riechers, & Lutz, 2008). Similar to injury and biomass reductions, the V5 application resulted in greater yield loss with  $\rm ED_{50}$  of 57 g ae  $\rm ha^{-1}$  at the V5 application versus 86 g ae  $\rm ha^{-1}$  at the V8 application for the white hybrid and 79 g ae  $\rm ha^{-1}$  versus 110 g ae  $\rm ha^{-1}$  for the yellow hybrid. The  $\rm ED_{5}$ , slope, and upper limit did not vary between growth stages.

# 3.2 | Hybrid

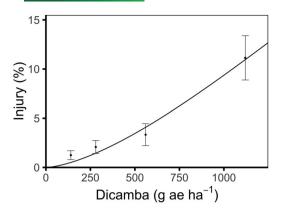
The white and yellow popcorn hybrids had similar injury response to glyphosate for all model parameters (Figure 1a). The ED $_{50}$  values at the V5 growth stage for white and yellow hybrid averaged 23 g ae ha $^{-1}$  compared with 73 g ae ha $^{-1}$  at the V8 growth stage (Table 2). The same trend was observed in the ED $_5$  values with injury averaging 5 and 46 g ae ha $^{-1}$ , at the V5 and V8 growth stages, respectively. The upper limit reached or exceeded 100% injury for both hybrids and was not allowed to exceed

<sup>&</sup>lt;sup>b</sup>Values in parentheses are standard error of parameter estimates.

<sup>&</sup>lt;sup>c</sup>Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where  $\alpha = .05$ .

<sup>&</sup>lt;sup>b</sup>Values in parentheses are standard error of parameter estimates.

 $<sup>^{</sup>c}$ Means presented within the same column for each herbicide with no common letter(s) are significantly different according to pairwise t-tests where  $\alpha = .05$ 



2964

FIGURE 2 Dose-response of two popcorn [Zea mays (L.) var. everta] hybrids (VWP111, white kernel color; and VYP315, yellow kernel color; Conagra Brands) at two growth stages (five-leaf stage and eight-leaf stage) to dicamba based on visual assessment of injury combined across years in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, Nebraska in 2017 and 2018. Regression lines represent the fit of a three-parameter log-logistic model. Error bars represent standard error of treatment means.

100%. With field corn, Brown et al. (2009) reported 3–16% conventional field corn injury at 14 DAT from 100 g ae ha<sup>-1</sup> of glyphosate applied at the V4–V5 growth stages. In sweet corn, glyphosate applied at 92 g ae ha<sup>-1</sup> resulted in 1–38% crop injury depending on carrier volume (12–281 L ha<sup>-1</sup>) at 14 DAT (Banks & Schroeder, 2002). Similarly, Ellis et al. (2002) reported 33–51% conventional field corn injury at 14 DAT from 140 g ae ha<sup>-1</sup> of glyphosate applied at the V6 growth stage. Glyphosate at 160 g ae ha<sup>-1</sup> resulted in 43% injury at 14 DAT when applied at the V6–V8 conventional field corn growth stages (Buehring et al., 2007).

Biomass reduction was greater in white popcorn hybrid than yellow hybrid when glyphosate was applied at the V5 growth stage; however, at the V8 growth stage, biomass reduction was similar between the two hybrids (Figure 1c). At the V5 growth stage, the ED<sub>50</sub> was 51 and 62 g ae ha<sup>-1</sup> for white and yellow popcorn hybrids, respectively (Table 3). The ED<sub>5</sub>, slope, and upper limit were similar for both hybrids within growth stage. Brown et al. (2009) reported 19% conventional field corn biomass reduction at 42 DAT from glyphosate at 100 g ae ha<sup>-1</sup> applied at the V4–V5 growth stages, which was substantially less than observed from popcorn in this research. Similarly, Banks and Schroeder (2002) reported sweet corn fresh weight reduction varying from 0 to 40% at 14 DAT with glyphosate applied at 92 g ae ha<sup>-1</sup>.

Popcorn height reduction following glyphosate application was not different between the two hybrids (Figure 1e; Table 4). Brown et al. (2009) reported 19% height reduction at 28 DAT from 100 g ae  $\text{ha}^{-1}$  of glyphosate applied at V4-V5

stage. Similarly, Ellis et al. (2002) reported 28–45% height reduction at 14 DAT from 140 g ae ha<sup>-1</sup> of glyphosate. Generally, less reduction in conventional field corn height has been reported in the literature than those observed at similar glyphosate rate in popcorn in this research. This might be because that popcorn is more sensitive to herbicides and tends to have shorter and thinner stalks compared to field corn.

Popcorn yield loss from glyphosate did not vary between hybrids (Figure 1g; Table 5). Brown et al. (2009) reported 9–31% conventional field corn yield loss from 100 g ae ha<sup>-1</sup> of glyphosate applied at the V4–V5 stages. Similarly, Ellis et al. (2002) reported 41–62% yield reduction from 140 g ae h<sup>-1</sup> of glyphosate applied at V6 field corn growth stage. Banks and Schroeder (2002) reported 0–51% loss in marketable sweet corn ears with glyphosate applied at 92 g ae ha<sup>-1</sup> when 20- to 25-cm tall. Thus, by comparing results of this research with scientific literature on conventional corn and sweet corn, it is evident that popcorn is more sensitive to glyphosate; therefore, care should be taken to avoid drift or tank contamination of glyphosate in popcorn fields to avoid yield loss.

A premix of 2,4-D choline/glyphosate applied to the white popcorn hybrid resulted in greater injury than the yellow hybrid at both growth stages (Figure 1b). The ED<sub>50</sub> values for the white and yellow hybrids at the V5 growth stage was 35 and 72 g ae ha<sup>-1</sup>, respectively (Table 2). The  $ED_{50}$  at the V8 growth stage was 77 and 98 g ae  $ha^{-1}$  for the same respective hybrids. The same trend occurred for the ED<sub>5</sub> values. No differences in model slope or upper limit (100%) occurred between hybrids within growth stages. In glyphosate-resistant field corn, 2,4-D choline/glyphosate applied at 1,720 g ae ha<sup>-1</sup> resulted in 0-63% injury at 7 DAT, with the predominate injury symptoms being fused brace roots, and 63% injury resulting from a location where the corn was at the V4 growth stage at the time of application (Ford et al., 2014). The highest rate of 2,4-D choline/glyphosate applied in this study was 550 g ae  $ha^{-1}$  (0.25×) which primarily resulted in glyphosate injury symptoms in both popcorn hybrids.

Similar to biomass reduction from glyphosate, 2,4-D choline/glyphosate resulted in greater biomass reduction in the white popcorn hybrid at the V5 growth stage and no difference between two hybrids at the V8 growth stage (Figure 1d). At the V5 stage, the  $\rm ED_5$  were 10 and 34 g ae ha<sup>-1</sup>, and the  $\rm ED_{50}$  were 47 and 109 g ae ha<sup>-1</sup> for the white and yellow hybrids, respectively (Table 3). The slope and upper limit were similar for both hybrids at both the V5 and V8 growth stages.

Similar to glyphosate, 2,4-D choline/glyphosate resulted in no differences in height reduction between hybrids (Figure 1f; Table 4). Popcorn yield loss from applications of 2,4-D choline/glyphosate followed similar trends to that

TABLE 6 Correlation coefficients and significance levels between popcorn yield loss and popcorn injury ratings, biomass reduction, and height reduction of two popcorn [Zea mays (L.) var. everta] hybrids (VWP111, white kernel color, Conagra brand; VYP315, yellow kernel color, Conagra brand) at two growth stages (V5, five-leaf stage; V8, eight-leaf stage) to glyphosate and 2,4-D choline/glyphosate based on yield loss combined across years in field experiments conducted at the University of Nebraska–Lincoln, South Central Agricultural Laboratory near Clay Center, NE in 2017 and 2018

Herbicide	Stage	Hybrid	Injury	Biomass reduction	Heightr eduction
Glyphosate	V5	VWP111	.99***	.96***	.96***
		VYP315	.98***	.94***	.97***
	V8	VWP111	.80***	.85***	.70***
		VYP315	.81***	.80***	.78***
2,4-D choline/glyphosate	V5	VWP111	.92***	.93***	.94***
		VYP315	.89***	.88***	.91***
	V8	VWP111	.92***	.86***	.83***
		VYP315	.93***	.85***	.83***
Dicamba	combined	combined	.10NS	NA <sup>b</sup>	NA

<sup>\*\*\*</sup> Significant at the .001 probability level; NS, nonsignificant at  $\alpha = .05$ .

of glyphosate as the  $ED_5$  and  $ED_{50}$  did not differ between hybrids (Figure 1h; Table 5).

# 3.3 | Correlation analysis and model fits

Correlation analysis suggests that popcorn injury ratings and biomass reduction were strongly correlated (Table 6). Correlation to yield loss ranged from 80 to 99% for glyphosate and 2,4-D choline/glyphosate injury ratings at 21 DAT and 80 to 96% for biomass reduction at 70 DAT. Deeds et al. (2006) reported similar correlation between yield loss and glyphosate injury ratings in wheat. Popcorn height reduction was correlated less strongly with yield loss than injury or biomass reduction, ranging from 70 to 97%. The correlation between dicamba injury and yield loss was weak (10%) because no yield losses were detected. The three-parameter log-logistic model had an adequate fit for all glyphosate and 2,4-D choline/glyphosate treatments with low RMSE and high ME values, suggesting the data captured the range of effects expected with a typical sigmoidal shaped dose-response curve; however, there was a poor fit to dicamba injury because only slight injury occurred (Tables 2, 3, 4, and 5).

# 3.4 | Practical implications

Unintentional application, spray drift, or improper tank clean out are challenges for popcorn producers operating in landscapes dominated by herbicide-resistant crops (USDA NASS, 2019) such as glyphosate-resistant corn and soybean in Nebraska. In this research, a constant carrier

volume was used, 140 L ha<sup>-1</sup>, which provided estimates of the minimum or low end of injury, biomass reduction, height reduction, and yield loss when considering drift rates. A proportional carrier volume to the herbicide rate results in greater herbicide effect (Banks & Schroeder, 2002; Smith, Ferrell, Webster, & Fernandez, 2017). Herbicide concentration in a drift event can be much less than the lowest dose tested in this study (0.03x). The growth stage of popcorn when lowest doses  $(0.03\times)$  of glyphosate or 2,4-D choline/glyphosate were applied was a critical factor for the level of injury and yield loss. With 0.03× of the labeled rate of glyphosate applied at the V5 growth stage, both hybrids resulted in >75% injury and yield loss; however, <15% injury and <45% yield loss when this rate of glyphosate was applied at the V8 growth stage. Similarly,  $0.03 \times$  of 2,4-D choline/glyphosate resulted in > 5% injury and >40% yield loss when applied at the V5 growth stage and <45% injury and <40% yield loss when applied at the V8 growth stage. Although the white and yellow popcorn hybrids tested in this research resulted in ≤11% injury and 0% yield loss with dicamba applied at 1,120 g as  $ha^{-1}(2x)$ , they resulted in nearly 100% yield loss with glyphosate as low as 105 g ae ha<sup>-1</sup> (0.063 $\times$ ) and 2,4-D choline/glyphosate as low as 275 g ae  $ha^{-1}$  (0.125×). The 2,4-D choline in the 2,4-D choline/glyphosate premix did not contribute noticeably to popcorn injury. Glyphosate is 51.5% of the total acid equivalent rate of 2,4-D choline/glyphosate; therefore, 275 g ae ha<sup>-1</sup> 2,4-D choline/glyphosate (0.125x) has 142 g ae ha<sup>-1</sup> of glyphosate. By comparing the injury curves from glyphosate and 2,4-D choline/glyphosate at 105 g ae ha<sup>-1</sup> of the glyphosate and 105 g ae ha<sup>-1</sup> of the glyphosate portion in 2,4-D choline/glyphosate, the majority if not all of the injury observed in popcorn treated

<sup>&</sup>lt;sup>a</sup>Because there was no difference, data of V5 and V8 growth stages were combined for dicamba.

<sup>&</sup>lt;sup>b</sup>NA, not applicable.

Agronomy Journal BARNES ET AL.

with 2,4-D choline/glyphosate was from the glyphosate portion of the premix. At 105 g ae ha<sup>-1</sup> glyphosate popcorn injury ranged from 87 to 95% with 2,4-D choline/glyphosate and from 89 to 97% with glyphosate alone. Dicamba is labeled for use in popcorn up to V5 growth stage; thus, potential drift of dicamba should not pose a risk to popcorn production. As of now, 2,4-D choline is not labeled for POST application in popcorn (Corteva Agriscience, 2017a). As glyphosate is applied to 90 and 240% (multiple applications) of corn and soybean fields in the United States (Kniss, 2017), there is great potential for application mistakes involving glyphosate. An increase in 2,4-D choline and 2,4-D choline/glyphosate application can be expected with the 2018 commercialization of 2,4-D choline/glyphosate/glufosinate-resistant corn (Enlist E3, Dow AgroSciences, Indianapolis, IN) and Enlist E3 soybean commercialized in 2019. Field corn and popcorn are nearly indistinguishable from each other during vegetative stages. This means that communication with applicators and agronomists must be maintained to avoid application mistakes to reduce off-target injury.

2966

The differences observed between white and yellow popcorn herbicide sensitivity in this study are inconclusive. With some measurements, the white popcorn hybrid (VWP111) was more sensitive to the tested herbicides than the yellow hybrid (VYP315), and with other measurements, there were no differences. In an experiment evaluating the response of two white and six yellow commercially available popcorn hybrids to several PRE and POST herbicides, Barnes et al. (2019) did not observe differences in injury between yellow and white popcorn hybrids. Although there is a perception that white popcorn is more sensitive to herbicides than yellow popcorn, there is only a single gene, Y1, responsible for yellow or white kernel color in maize (Buckner, Miguel, Janick-Buckner, & Bennetzen, 1996; Ford, 2000). This is the first research to characterize white and vellow popcorn hybrid response to glyphosate, 2,4-D choline/glyphosate, and dicamba in the published literature. This research will play an important role in educating applicators and agronomists about sensitivity of white and yellow hybrids to glyphosate, 2,4-D choline/glyphosate, and dicamba.

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