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Enlist™ corn tolerance to preemergence and postemergence applications of synthetic auxin and ACCase-inhibiting herbicides

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Abstract

Allowing the use of two additional modes of action (MOAs), Enlist™ corn is a novelty in the continuum of herbicide-resistant crop development efforts that have occurred since the 1990s. Knowledge of Enlist corn tolerance to labeled herbicides and other herbicides within the same MOA for various use and/or exposure scenarios is not well established. Four site-year field experiments for preemergence (PRE) and postemergence (POST) applications were conducted at sites in Fayetteville (2021 and 2022) and Tillar (2020 and 2021), Arkansas, to evaluate Enlist corn response following PRE or POST applications of synthetic auxin herbicides or those that inhibit acetyl-CoA carboxylase (ACCase). A non-Enlist and an Enlist corn hybrid were used for each herbicide treatment to establish differential tolerance. Injury response to PRE application varied among site-years; clethodim was the only herbicide that occasionally caused significant (7% to 17%) injury to Enlist corn. None of the PRE treatments affected plant height, stand, or yield of Enlist corn; these responses were generally similar or better for Enlist corn compared to non-Enlist corn. Enlist corn showed significant injury to POST applications of florpyrauxifen-benzyl (>10%), fluazifop-P-butyl and quizalofop-P-ethyl (>5%), and clethodim and sethoxydim (>75%) 1 wk after application (WAA). These initial injury responses to clethodim and sethoxydim were generally reflected in Enlist corn yield; however, the minimal injury from fluazifop-P-butyl and quizalofop-P-ethyl did not affect yield. Injury to non-Enlist corn with POST-applied ACCase-inhibiting herbicides 2 WAA was >80%, resulting in a proportionate yield reduction. Even though florpyrauxifen-benzyl caused more initial injury to non-Enlist corn, yield reduction in non-Enlist corn was occasionally less than of Enlist corn, with both hybrids experiencing >75% yield reduction. In summary, Enlist corn may occasionally show transient injury even to labeled herbicides when applied POST, and even though the injury from florpyrauxifen-benzyl is initially mild, it nonetheless results in substantial yield loss.

Introduction

Enlist™ corn (*Zea mays* L.), which was recently introduced, has transgenes that confer resistance to herbicides from two modes of action (MOAs). This novel technology for weed management in corn is restricted to only one herbicide from each acetyl-CoA carboxylase (ACCase)–inhibitor and synthetic auxin MOA. Quizalofop-P-ethyl and 2,4-dichlorophenoxyacetic acid (2,4-D) are the only herbicides from these MOAs that are labeled for use on Enlist corn crops. This metabolism-based aryloxyalkanoate dioxygenase (AAD-1) trait in Enlist corn provides resistance to specific chemical classes of the herbicides: the aryloxyphenoxypropionate class of ACCase-inhibiting herbicides (also known as FOPs) and the phenoxycarboxylic class of synthetic auxins (Wright et al. 2010). Enlist corn hybrids are typically stacked with glyphosate and/or glufosinate resistance traits. Coupled with the improved formulation of 2,4-D (choline salt) and the other stacked resistance traits, this technology offers highly efficacious weed management solutions in corn production systems compared to previously available technologies.

A wide array of weed management solutions are available for corn (Barber et al. 2022); however, management of volunteer corn within the crop can be challenging. With the widespread use of herbicide-resistant (HR) corn technology in the United States, there has been a concomitant increase in the presence of overwintered volunteer HR corn in rotated crops (Davis et al. 2008), raising management difficulties (Chahal and Jhala 2015; Marquardt et al. 2012). Successful use of both FOPs and cyclohexanedione (also known as DIMs) families of ACCase-inhibiting herbicides for managing volunteer HR corn in broadleaf crops, especially soybean [*Glycine max* (L.) Merr], has been extensively demonstrated (Alms et al. 2016; Chahal and Jhala 2015; Deen et al. 2006; Kniss et al. 2012). Recently developed by Corteva Agriscience and possessing transgenic resistance to FOP herbicides, Enlist corn allows the use of quizalofop-P-ethyl (a FOP herbicide) to control volunteer non-Enlist corn among Enlist corn plantings. This opportunity for selective in-season management of volunteer non-Enlist corn in corn is an unprecedented use for ACCase-inhibiting herbicides in the crop. The pro-herbicide quizalofop-P-ethyl passively penetrates corn cuticle tissue and is subsequently bioactivated to the active quizalofop-acid form by carboxyesterases (Cummins and Edwards 2004; Haslam et al. 2001). As conferred by the *AAD-1* gene (*TfdA* homologs), its ability to rapidly metabolize absorbed quizalofop-P-ethyl (and other FOP herbicides) differentiates Enlist corn from non-Enlist corn.

AAD-1 in Enlist corn can simultaneously degrade 2,4-D. 2,4-D, the first selective, broad-spectrum broadleaf herbicide used in corn for more than seven decades, can cause corn injury (Ruen et al. 2017). Despite limited translocation of this systemic herbicide in corn and ability of the crop to metabolize (conjugate) 2,4-D (Fang and Butts 1954; Montgomery et al. 1971), only the commercially deployed AAD-1 trait provides robust resistance to 2,4-D in Enlist corn permitting higher 2,4-D use rates across a wider application window (Ruen et al. 2017; Wright et al. 2010).

Several other ACCase-inhibiting and synthetic auxin herbicides, including some that were recently developed, are commonly used in cropping systems in the mid-South (Barber et al. 2022). Fluazifop-P-butyl (a FOP), clethodim, sethoxydim (DIMs), florpyrauxifen-benzyl, and halauxifen-methyl (arylpicolinates in the family of synthetic auxins) are used in cotton (*Gossypium hirsutum* L.), soybean, and/or rice (*Oryza sativa* L.) crops in the region. Enlist being the new corn HR trait and the first-ever commercialized HR trait conferring resistance to herbicides from synthetic auxins and ACCase-inhibiting MOAs must be assessed for its safety for prospective herbicide use or exposure scenarios. The objective of this research was to evaluate the response of Enlist corn in comparison with glyphosate-resistant (non-Enlist) corn to synthetic auxins and ACCase-inhibiting herbicides applied preemergence (PRE) and postemergence (POST) for potential implications for Enlist corn safety under normal and replanting situations, and volunteer corn control.

Materials and Methods

Experimental Setup

Four field experiments were conducted in Arkansas at two locations (sites) over 2 yr: Fayetteville (2021 and 2022) and Tillar (2020 and 2021). Soil type, herbicide application dates, cumulative precipitation, and general weather conditions (seasonal and following treatment applications) for each site-year are presented in Table 1 and Figure 1. The experiments were established as a two-factor (trait and herbicide) randomized complete block design with four replications. Corn hybrids (trait; Mycogen UNI 14D38 Enlist corn and 6252RIB non-Enlist corn in Fayetteville, and Mycogen UNI 14D38 or B10Z78SXE Enlist corn and Pioneer P1197YHR non-Enlist corn in Tillar) were planted 2.5 to 3 cm deep at a seeding rate of 69,160 to 86,450 seeds ha^{-1} with a 76- to 97-cm-wide row spacing, depending on location. Plot sizes were four rows wide and 9 m long.

Except for florpyrauxifen-benzyl, each herbicide was applied at a single rate in both the PRE and POST application experiments (Table 2). Herbicides were applied to two center rows of corn in four-row plots at planting (PRE) or at the two- to three-leaf stage (POST). A nontreated control was included for each corn hybrid. Herbicide treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 166 kPa fitted with AIXR 110015 flat-fan nozzles (TeeJet®; Spraying Systems Co., Wheaton, IL).

At the Fayetteville location, weeds were controlled with application of glyphosate (Roundup PowerMAX®; Bayer CropScience LP, Research Triangle Park, NC) at 1,260 g ae ha^{-1} plus halosulfuron-methyl at 70 g ai ha^{-1} (Permit®; Gowan Company LLC, Yuma, AZ) to three-leaf corn followed by a premix of *S*-metolachlor + mesotrione + bicyclopyrone + atrazine at 1,500 + 166 + 41 + 700 g ai ha^{-1}, respectively (Acuron®; Syngenta Crop Protection, LLC, Greensboro, NC) and glyphosate (Roundup PowerMAX®) at 1,260 g ae ha^{-1}. At the Tillar location, atrazine at 2,240g ai ha^{-1} (Attrex®; Syngenta Crop Protection, LLC) plus glyphosate at 1,260 g ae ha^{-1} (Roundup PowerMAX®) were applied to the entire experimental area. Fertility and pest management were maintained throughout the experiment based on University of Arkansas System Division of Agriculture Cooperative Extension Service recommendations (Faske et al. 2022; Studebaker et al. 2022).

Data Collection

Visible injury (a composite assessment of chlorosis, necrosis, and stunting) was rated at 3 and 5 wk after application (WAA) for the PRE treatments, and 1 and 2 WAA for the POST treatments. Visible injury ratings were based on a scale of 0% (no injury, nontreated control) to 100% (complete plant death). Plant height (four random plants per plot) was measured at the Tillar location for both PRE and POST experiments, and plant stand was measured at the Fayetteville location for PRE experiments. Two center rows of corn were harvested (except for Tillar 2021) at crop maturity using a plot combine, and grain yield was adjusted to 15% moisture. Corn was accidentally harvested by the farmer at the Tillar location in 2021. Plant height, stand, and yield data were converted to a percentage of respective traits' nontreated control.

Statistical Analysis

Statistical analysis was performed using R statistical software version 4.2.2 (R Core Team 2022) using the GLMMTMB package (function *glmmTMB*; Brooks et al. 2017). Herbicide-by-trait-by-site interactions were evaluated for all combinations of sites, and if they were nonsignificant, data were pooled for subsequent analysis. In the models, the interaction of herbicide and trait was considered a fixed effect, and block was a random effect.

Corn injury, plant height, plant stand, and yield data were fit to generalized linear mixed-effect models using the *glmmTMB* function with gaussian (link = "identity") error distributions (Stroup 2015). Additionally, the model for yield data for the POST application was fitted with zero-inflation error distribution. Selection for final *glmmTMB* models was based on Akaike information criterion values and/or restricted maximum likelihood criterion at convergence.

The ANOVA was performed using the CAR package (Fox and Weisberg 2019). For *glmmTMB* models, ANOVA was conducted with Type III Wald chi-square tests. After conducting ANOVA, treatment estimated marginal means (Searle et al. 1980) were separated using the EMMEANS package (Lenth 2022) and MULTCOMP package (Hothorn et al. 2008). Estimated marginal means included post hoc Tukey P-value adjustments and Sidak method confidence-level adjustments, with compact letter display generated via the *multcomp:cld* function.

Table 1. Site-year, soil type, herbicide application dates, cumulative precipitation, and weather conditions.[a]

		Date of treatment application			Moisture and temperature conditions following treatment application[d]	
Site, year	Soil type	PRE[b]	POST	Precipitation[c]	PRE	POST
				cm		
Fayetteville, 2021	Captina silt loam	April 8	May 18	67	Moist, cool	Moist, moderate
Fayetteville, 2022	Captina silt loam	April 27	May 27	70	Wet, moderate	Moist, warm
Tillar, 2020	Hebert silt loam	May 12	June 3	81	Dry, warm	Moist, warm
Tillar, 2021	Hebert silt loam	May 8	June 4	76	Moist, moderate	Wet, warm

[a]Abbreviations: AT, average temperature; CT, cumulative precipitation; POST, postemergence; PRE, preemergence.
[b]Corn planted on the same day.
[c]Cumulative of daily amount beginning April 1 through September 30.
[d]Represents the period of 15 d following application: cool, AT less than 15 C; moderate, AT 15 C to 22 C; warm, AT above 22 C; dry, CP for 15 d less than 5 cm; moist, CP 5 cm to 10 cm; wet,10 cm or more. Refer to Figure 1.

Table 2. Herbicide information.[a]

Herbicide	Trade name	Manufacturer	Rate[b]	Is the herbicide labeled for use in Enlist corn?	Rotation/replant interval for non-Enlist corn
			g ai ha^{-1}		d
Synthetic auxins					
2,4-D choline	Enlist One®	Corteva Agriscience LLC	1,065	Yes	0
Florpyrauxifen-benzyl	Loyant®	Corteva Agriscience LLC	15[c,d]	No	0[f]
Halauxifen-methyl	Elevore™	Corteva Agriscience LLC	6[e]	No	15
ACCase-inhibitors -FOPs					
Fluazifop-P-butyl	Fullisade®DX	Syngenta Crop Protection, LLC	210	No	60
Quizalofop-P-ethyl	Provisia®	BASF Corporation	115	Yes	120
ACCase-inhibitors -DIMs					
Clethodim	Select Max®	Valent U.S.A. LLC	136	No	6
Sethoxydim	Poast Plus®	BASF Corporation	210	No	30

[a]Abbreviations: DIMs, cyclohexanedione herbicides; FOPs, aryloxyphenoxypropionate herbicides.
[b]All herbicides were applied with 1% vol/vol crop oil concentrate.
[c]Applied postemergence only.
[d]1× rate.
[e]Applied at preemergence only.
[f]Drift warning from adjacent application and no residual activity.

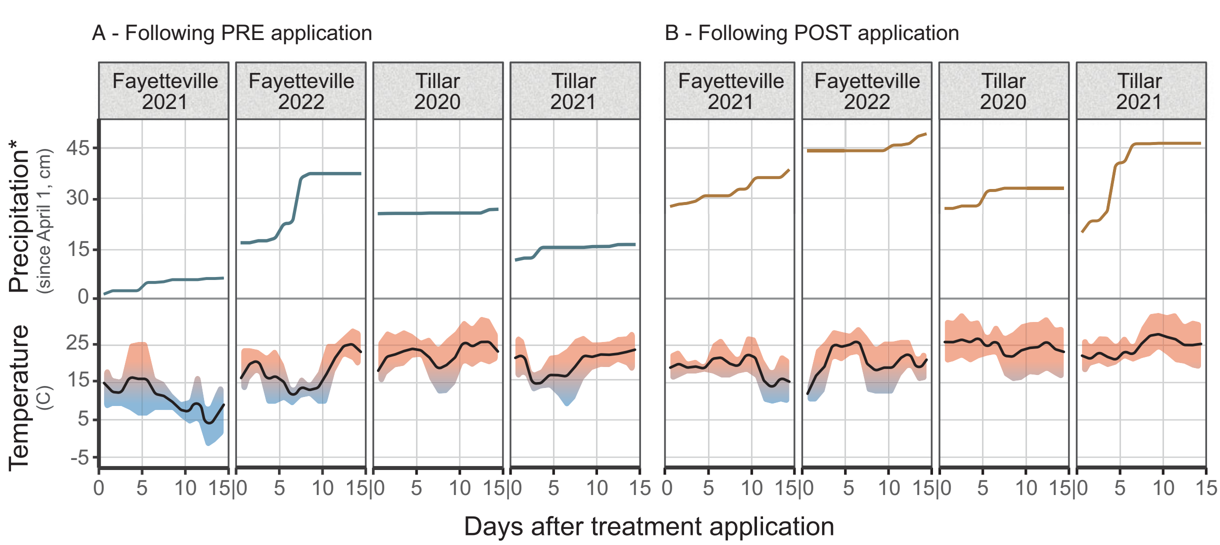

Figure 1. Daily cumulative precipitation and air temperature for the period of 15 d following (A) preemergence (PRE) and (B) postemergence (POST) applications at sites in Fayetteville and Tillar, Arkansas, in 2020, 2021 and/or 2022. In the lower panel, the upper boundary = maximum daily temperature, the lower boundary = minimum daily temperature, and the line = average daily temperature. The asterisk (*) indicates cumulative daily precipitation.

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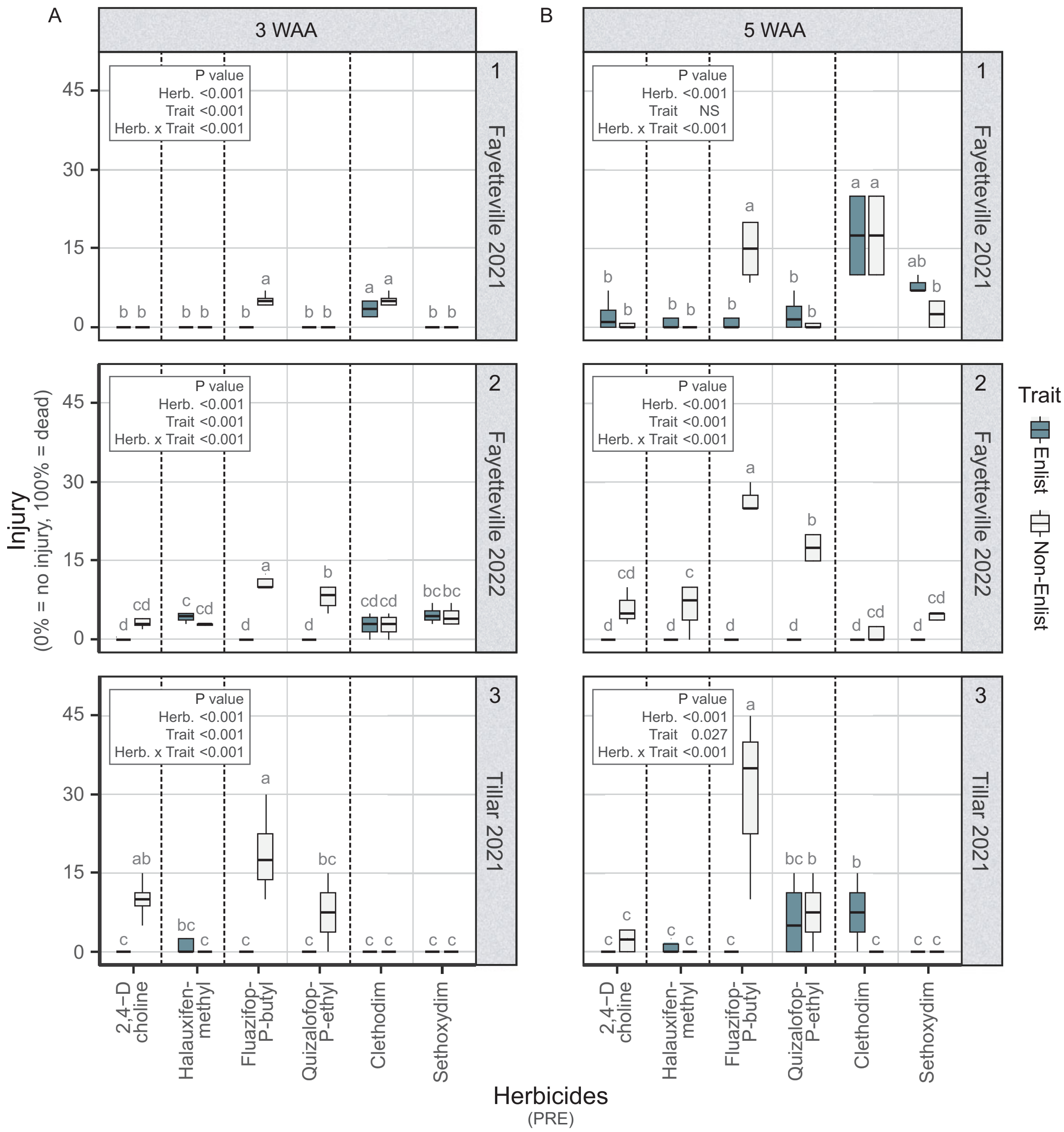

Figure 2. Injury response of Enlist corn to preemergence (PRE) application of synthetic auxin and ACCase-inhibiting herbicides (A) 3 wk after application (WAA) (B) 5 WAA at the Fayetteville site (2021 and 2022), and at the Tillar site (2021) in Arkansas. Injury is expressed as a percent of nontreated corn (0% = no injury, 100% = plant death). ANOVA output of a generalized linear mixed model (herbicide and trait as fixed factors, and block as random factor) are shown at the top left corner of each plot. Means with the same letter within a plot are not significantly different ($P < 0.05$ using Tukey's honestly significant difference test). Herb. Indicates herbicide; NS indicates nonsignificant.

Results and Discussion

Enlist Corn Response to PRE Application

Corn planting for the Fayetteville site in 2021 occurred earlier than the other site-years (Table 1). Corn planting in Arkansas begins the first week of April, and most planting is completed by the third week of May (USDA-NASS 2022). The weather conditions (temperature and/or precipitation) following application, particularly for PRE application, varied among the sites (Figure 1), which may have caused some variability in corn response to the herbicide treatments. The potential attribution of corn response to weather conditions is discussed as needed.

Injury

Corn injury at 3 WAA with PRE treatments varied among sites resulting in herbicide-by-trait-by-site interactions ($P < 0.001$). Effects of herbicide, trait, and their interaction were significant for three sites, but not Tillar 2020 (Figure 2A). At the Fayetteville site in 2021, only clethodim caused injury to Enlist corn (5%; Figure 2A1), and the level of injury was similar for non-Enlist corn. A similar level of injury with clethodim was observed at the Fayetteville site in 2022 (Figure 2A2). In addition to clethodim, halauxifen-methyl and sethoxydim caused injury (>5%) at the Fayetteville location in 2022, and the level of injury was similar for non-Enlist corn with these herbicides; but injury from these herbicides was not present at Tillar sites (Figure 2A3; data not shown for Tillar 2020). While Enlist corn did not show any injury with 2,4-D, fluazifop-P-butyl, or quizalofop-P-ethyl at any sites, injury to non-Enlist corn was frequently pronounced and ranged from 5% to 20% and was usually greater with fluazifop-P-butyl.

By 5 WAA, non-Enlist corn injury across sites was generally more pronounced with most herbicides that had caused injury

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at 3 WAA (Figure 2A and B). With interaction of trait-by-time of evaluation being significant (P < 0.001), averaged across herbicides and sites, injury to Enlist corn remained similar, whereas injury to non-Enlist corn increased by three percentage points at 5 WAA from injury at 3 WAA (P < 0.05). Enlist corn at the Fayetteville location showed >15% injury with clethodim and at least 10% injury with sethoxydim in 2021 (Figure 2B1), whereas corn had recovered from all initial injuries by 5 WAA in 2022 (Figure 2B2). For all herbicides at the Tillar location in 2020, injury was typically nonsignificant at 5 WAA, like the results at 3 WAA (data not shown); nevertheless, >5% injury was visible with quizalofop-P-ethyl or clethodim for Enlist corn in 2021 (Figure 2B3).

The discrepancies in corn injury response to these PRE-applied herbicides at different sites is likely the result of weather conditions following the applications (Figure 1). Precipitation, soil moisture, and temperature may explain the variations observed considering the resistance mechanism is metabolism-based and PRE-applied herbicides need to be activated. Soil moisture content has long been known to affect herbicide partitioning into adsorbed and solution phases (Green and Obien 1969) and to modulate herbicide transport within the soil-plant system, resulting in differential phytotoxicity (Walker 1971). While the effect of soil moisture varies from herbicide to herbicide (Ferreira et al. 2021), the pronounced injury at 5 WAA in 2021 at the Fayetteville location with clethodim may be attributed to moist, cool conditions following herbicide application (Figures 1 and 2B1). A high amount of rainfall that coincided with the crop emergence time at the Fayetteville site in 2022 may have contributed to overall greater injury 3 WAA. In contrast, relatively dry conditions at the Tillar location in 2020 likely safeguarded emerging corn plants from exposure to herbicides in the soil solution (Figure 1).

Plant Height and Stand

Plant height (at 6 WAA) and stand (at 5 WAA) were recorded at Tillar and Fayetteville sites, respectively (Figure 3). For height data, there was three-way interaction of herbicide-by-trait-by-year (P < 0.001). The effect of herbicide or its interaction with trait was not significant in 2020, however, the effect of trait was still significant, with halauxifen-methyl causing 10% height reduction of non-Enlist corn and with overall (across herbicides) greater height of Enlist corn compared to non-Enlist corn (P < 0.001; Figure 3A). In 2021, the herbicide treatments or their interaction with trait was significant, with fluazifop-P-butyl resulting in >10% height reduction of non-Enlist corn (Figure 3B).

Similarly for plant stand at the Fayetteville location, herbicide-by-trait-by-year interaction was significant (P < 0.001). Effects of herbicide, trait, and their interaction were significant in 2021 (Figure 3C), whereas none of the effects were significant in 2022 (data not shown). In 2021, herbicide treatments caused a 4% reduction in non-Enlist corn stands on average, compared to the nontreated control. However, clethodim resulted in a significant (10% to 12%) stand reduction of Enlist corn compared to FOP herbicides or the nontreated control.

Yield

Because herbicide-by-trait-by-site interaction did not occur for the Fayetteville (2022) and Tillar (2020) yields (P > 0.05), data were pooled and analyzed separately from Fayetteville (2021) data. Yield data for Tillar (2021) were not collected. Averaged over two sites (Fayetteville 2022 and Tillar 2020), no Enlist treatments resulted in any yield reduction. With the absence of herbicide-by-trait interaction (P = 0.476), Enlist corn produced 8% greater yield,

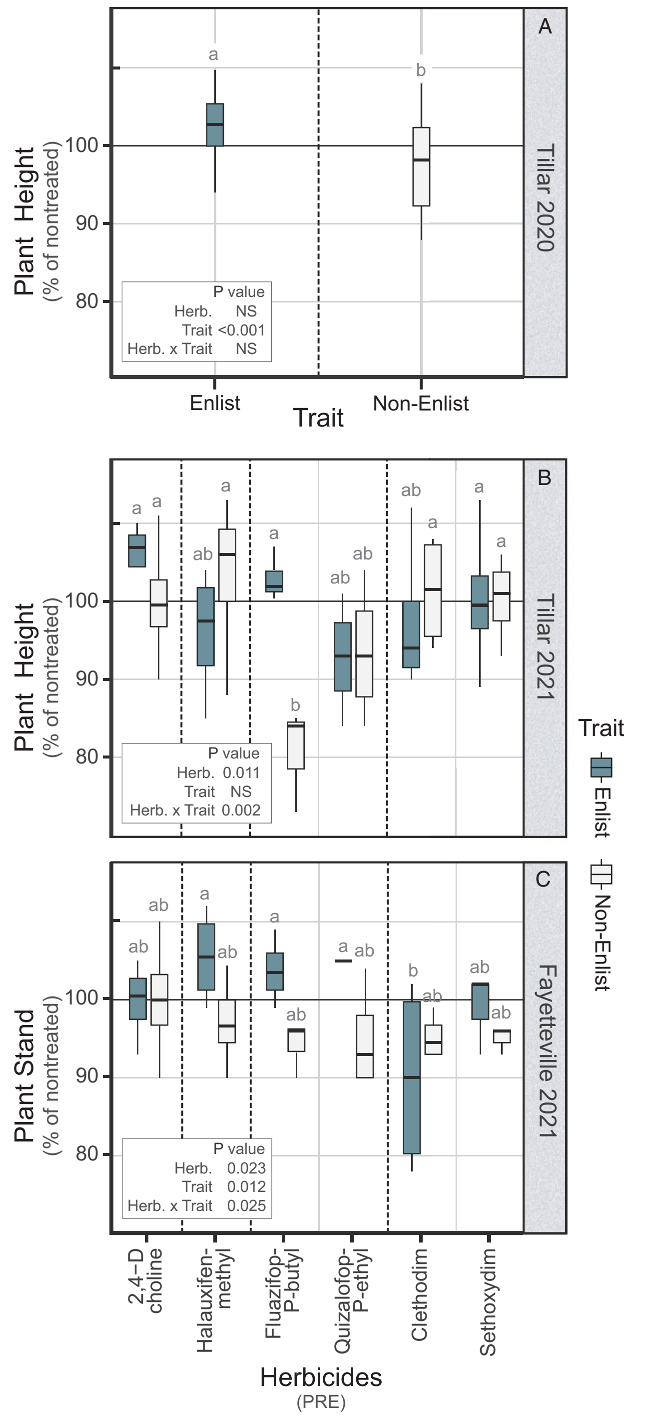

Figure 3. (A and B) Plant height 6 wk after application (WAA) and (C) plant stand 5 WAA of Enlist corn as affected by preemergence (PRE) application of synthetic auxin and ACCase-inhibiting herbicides at the Fayetteville site (2021), or the Tillar site (2020 and 2021) in Arkansas. Height and stand are expressed as a percent of the nontreated control (100% = height or stand of nontreated control). ANOVA output of a generalized linear mixed model (herbicide and trait as fixed factors, and block as random factor) are shown at the bottom left corner of each plot. Means with the same letter within a plot are not significantly different (P < 0.05 using Tukey's honestly significant difference test). Herb. indicates herbicide; NS indicates nonsignificant.

on average, compared to non-Enlist corn (P = 0.039; Figure 4A). While yields were similar across non-Enlist treatments, grain yield following application of halauxifen-benzyl was 19% less for non-Enlist corn compared to the same treatment on Enlist corn (data

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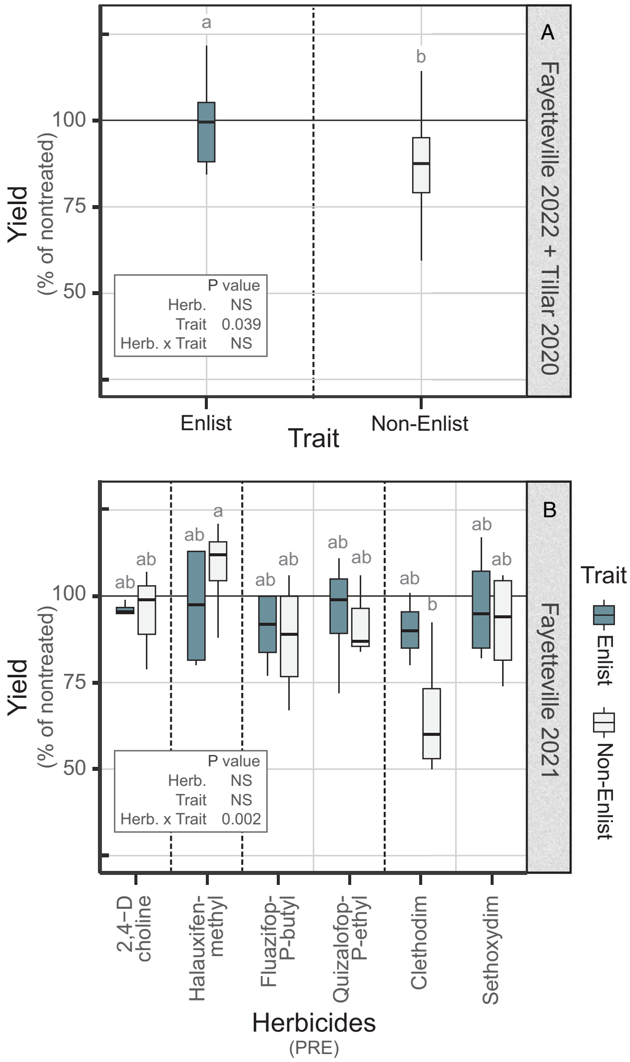

Figure 4. Yield of Enlist corn as affected by preemergence (PRE) application of synthetic auxin and ACCase-inhibiting herbicides at (A) the Fayetteville site (2022) and the Tillar site (2020), and (B) at the Fayetteville site (2021) in Arkansas. Yield is expressed as a percent of the nontreated control. ANOVA output of a generalized linear mixed model (herbicide and trait as fixed factors, and block as random factor) are shown at the bottom left corner of each plot. Means with the same letter within each plot are not significantly different ($P < 0.05$ using Tukey's honestly significant difference test). Herb. indicates herbicide; NS indicates nonsignificant.

not shown). These yield results generally corroborate well with the Fayetteville (2022) injury data (Figure 2B). Yield results from Tillar (2020) did not interact with yield results from Fayetteville (2022), despite the differentiated injury response between the two sites. The Tillar site received much less precipitation for an extended period following the herbicide application (Figure 1). The herbicide-by-trait interaction was the only significant effect for Fayetteville (2021) yield, and clethodim on non-Enlist corn was the only treatment that caused yield loss (>30%; Figure 4B). Despite some injury with clethodim and sethoxydim at 5 WAA (Figure 2B1), Enlist corn produced similar yields across herbicide treatments.

Weather conditions following the PRE application varied among the sites. Corn planting dates for the central United States are approximately 2 wk earlier than they were in the 1980s (Kucharik 2006), and in Arkansas, corn planting begins as early as April and concludes by the end of May (USDA-NASS 2022). Hence, corn at all sites was planted within the normal range. Daily air temperature following planting/herbicides application was low at the Fayetteville site in 2021. Greater injury observed with clethodim on both Enlist and non-Enlist hybrids may be attributed to cooler temperature because it can impede metabolism and sequestration of herbicides (Ghanizadeh and Harrington 2017; Kudsk and Kristensen 1992). For example, Robinson et al. (2015) reported that cooler temperatures caused crop injury to winter wheat (*Triticum aestivum* L.) after the application of 2,4-D alone or in combination with dichlorprop, as well as a mixture of dicamba, MCPA, and mecoprop. Additionally, cloudy days following application may have exposed clethodim to photodegradation to a lesser extent, allowing a significant proportion of herbicide to penetrate the soil. Kinetic data on photodegradation suggest that clethodim and sethoxydim are subject to rapid degradation after their application in the field (Sandín-España et al. 2015). Clethodim is very potent at a low rate, and if it is absorbed into grass plants, it shows a rapid photodegradation with half-lives from 19.3 min to 1.4 h in an aqueous environment (Villaverde et al. 2018). Like other DIM herbicides, clethodim is water-soluble and poorly adsorbed in soil (EFSA 2011). Thus, the role of photodegradation rate on the soil surface before its diffusion into the soil system in the phytotoxicity of PRE-applied clethodim cannot be ignored.

Besides occasional injury from PRE-applied DIM family of ACCase-inhibiting herbicides, Enlist corn height, stand, and yield were not affected by any of the herbicides from the two MOAs used in this study. Non-Enlist corn usually showed a similar response as Enlist corn, with instances of discrepancies such as reduced plant height with halauxifen-benzyl or fluazifop-P-butyl and reduced yield with clethodim. There is no or little published information regarding the response of Enlist corn, particularly to PRE-applied ACCase-inhibiting herbicides. In one study in Tennessee, Steckel et al. (2009) reported no injury from clethodim applied at 50 g ai ha^{-1} at 2 d after planting of replanted corn. The clethodim was applied at a much higher rate in this study (136 g ai ha^{-1}). Upon application to soil, several chemical characteristics of a herbicide such as solubility, soil organic carbon-water partitioning coefficient (K_{oc}), and half-life determine its potency to cause phytotoxicity, which is beyond the scope of this research and is not discussed further. Given the fact that results were not completely confirming among the sites, as well as the absence or presence of only a thin range of discrepancies between hybrids with or without the AAD-1 enzyme, it is difficult to conclude whether Enlist corn is any better than non-Enlist corn across environments in terms of its sensitivity to PRE-applied nonlabeled herbicides evaluated in this study. However, for the labeled herbicides, Enlist corn invariably showed no significant injury in contrast to non-Enlist corn, which showed frequent injury, especially with the FOP herbicides. This indicates that the AAD-1 enzyme and the degradation mechanism in Enlist corn are operative early in the growth stage.

Enlist Corn Response to POST Application

Injury

Corn injury data for POST applications were pooled across four sites for subsequent analysis because there was no herbicide-by-trait-by-site interaction ($P > 0.05$). For both the evaluation timings, all effects of herbicide, trait, and their interaction were significant (Figure 5). At 1 WAA, all the Enlist and non-Enlist POST treatments caused 5% or more crop injury, with clethodim and

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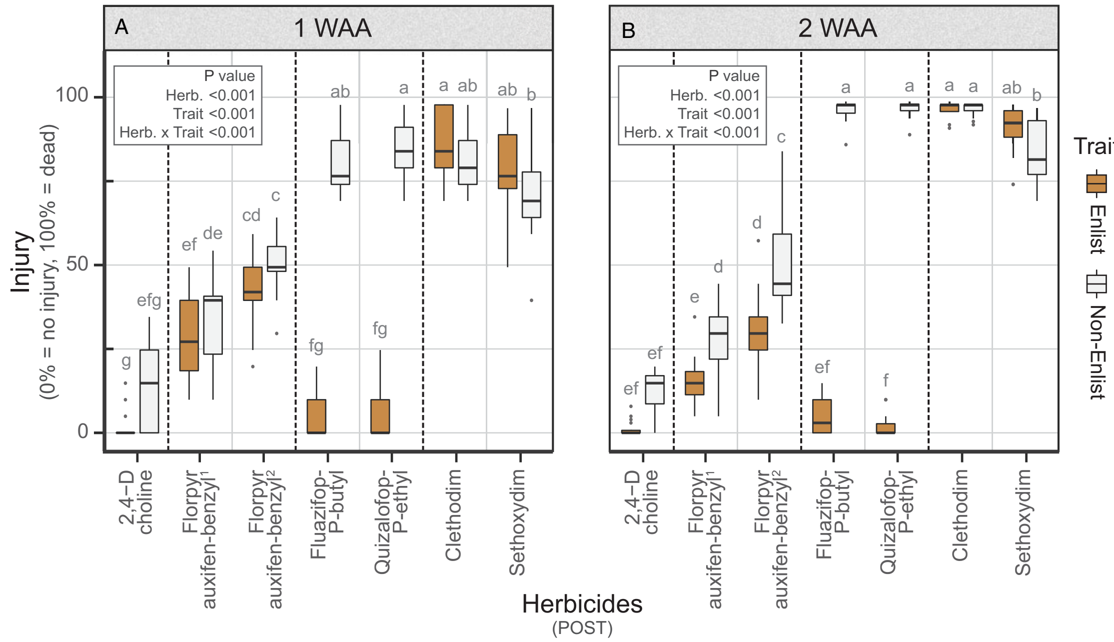

Figure 5. Injury response of Enlist corn to postemergence (POST) application of synthetic auxin and ACCase-inhibiting herbicides (A) 1 wk after application (WAA) and (B) 2 WAA. Data were pooled for four site-year experiments: Fayetteville (2021 and 2022) and Tillar (2020 and 2021) in Arkansas. Injury is expressed as a percent of the nontreated (0% = no injury, 100% = plant death). ANOVA output of a generalized linear mixed model (herbicide and trait as fixed factors, and block as random factor) are shown at the top left corner of each plot. Means with the same letter within a plot are not significantly different ($P < 0.05$ using Tukey's honestly significant difference test). [1]1× rate; [2]2× rate. Herb. indicates herbicide.

sethoxydim resulting in >65% injury (Figure 5A). The highly differentiated responses between Enlist and non-Enlist corn hybrids were observed with fluazifop-P-butyl or quizalofop-P-ethyl (>5% vs. >75%, respectively). Averaged across presence or absence of the AAD-1 trait, injury to corn from florpyrauxifen-benzyl was at least 25% for the 1× rate and >40% for the 2× rate. Despite instances of injury in some plots, Enlist corn did not exhibit injury with 2,4-D. Conversely, non-Enlist corn showed variable injury, ranging from no injury to 30% injury. At 2 WAA, Enlist corn had slightly recovered from the initial injury caused by all herbicides, except for clethodim (Figure 5B). Non-Enlist corn generally maintained a similar level of injury from synthetic auxin treatments, whereas injury with fluazifop-P-butyl, quizalofop-P-ethyl, or clethodim was more pronounced (>90% injury). Non-Enlist corn injury was greater than that of Enlist corn at both florpyrauxifen-benzyl rates (averaged across rates, 22% vs. 38% injury, respectively). Corn injury from sethoxydim was not as severe as from clethodim, especially in non-Enlist corn.

Plant Height

Corn height data for the Tillar 2020 and 2021 site-years were analyzed separately because they were recorded at different time points relative to the application time (Figure 6). Effects of herbicide, trait presence, and their interaction were significant in 2020 (Figure 6A). All herbicides applied to Enlist corn, except for 2,4-D and quizalofop-P-ethyl, reduced plant height by at least 10%, with clethodim and sethoxydim causing a >80% reduction. Height reduction for non-Enlist corn was overall greater ($P < 0.001$), but not significant compared to Enlist corn, except when fluazifop-P-butyl and quizalofop-P-ethyl were applied. Similar differentiation between the presence or absence of the AAD-1 trait was observed in 2021, with effects of herbicide, trait, and their interaction being significant (Figure 6B). Evaluated 1 wk earlier than in 2020, injury was, in general, greater for synthetic auxins and less for ACCase-inhibitors in 2021 compared to the injury levels in 2020.

Yield

Fayetteville yield data for 2 yr were pooled and analyzed separately from Tillar (2020) data since herbicide-by-trait-by-site interaction did not occur for those 2 yr ($P > 0.05$; Figure 7). Effects of herbicide, trait, and their interaction were significant for both Fayetteville and Tillar yields (Figure 7A and B). At the Fayetteville location, except for 2,4-D, fluazifop-P-butyl, or quizalofop-P-ethyl, all other herbicides reduced grain yield of Enlist corn, ranging from >75% with florpyrauxifen-benzyl to >95% with clethodim or sethoxydim. For Enlist corn, similar yield reductions with all the herbicides occurred, with additional yield loss (complete loss) from fluazifop-P-butyl and quizalofop-P-ethyl. Although the non-Enlist corn yield with 2,4-D was similar to the mean yield of Enlist corn for the same herbicide (i.e., 100%), the non-Enlist corn yield with 2,4-D was <100% (null hypothesis against 100, $P = 0.03$). Yield results were generally similar for Tillar, except for florpyrauxifen-benzyl and sethoxydim. With a 1× rate of florpyrauxifen-benzyl, the grain yield of Enlist corn was <75% of the nontreated, whereas it was at least half of the nontreated for non-Enlist corn. Sethoxydim did not result in a complete yield loss of non-Enlist corn.

Injury response to the POST applications of herbicides was consistent among the sites. Neither 2,4-D nor FOP herbicides caused significant injury to Enlist corn when applied POST. Injury development with DIM herbicides was rapid in Enlist corn and was generally similar to that of non-Enlist corn, implying that DIM herbicides can be successfully used to control volunteer corn.

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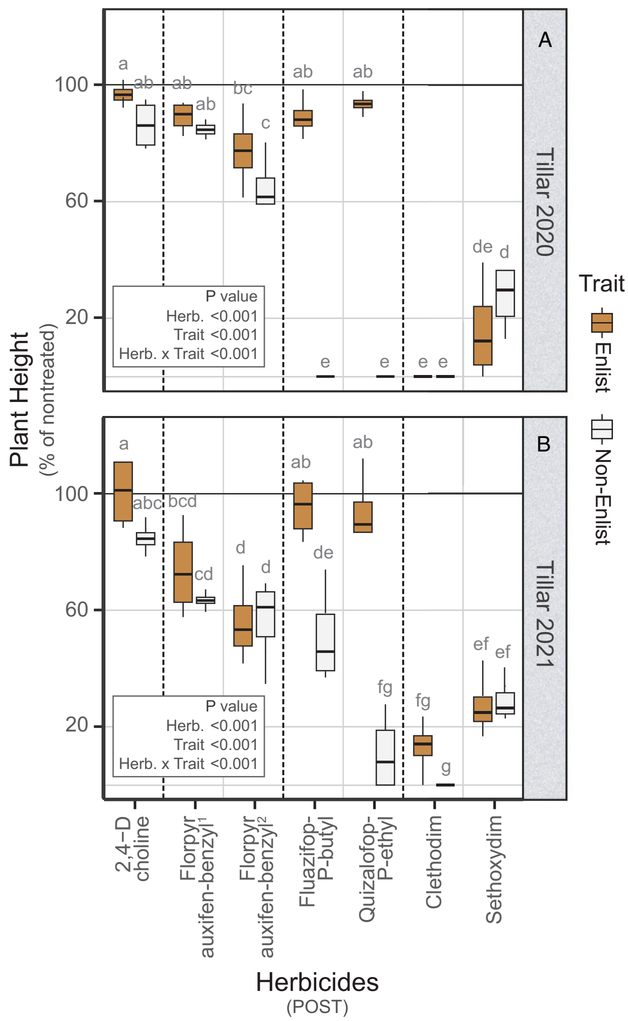

Figure 6. Height of Enlist corn as affected by postemergence (POST) application of synthetic auxin and ACCase-inhibiting herbicides at the Tillar location in Arkansas in (A) 2020, 3 wk after application (WAA) and (B) 2021, 2 WAA. Height is expressed as a percent of the nontreated control (100 = height of nontreated control). ANOVA output of a generalized linear mixed model (herbicide and trait as fixed factors, and block as random factor) are shown at the bottom left corner of each plot. Means with the same letter within a plot are not significantly different ($P < 0.05$ using Tukey's honestly significant difference test). [1]1× rate; [2]2× rate. Herb. indicates herbicide.

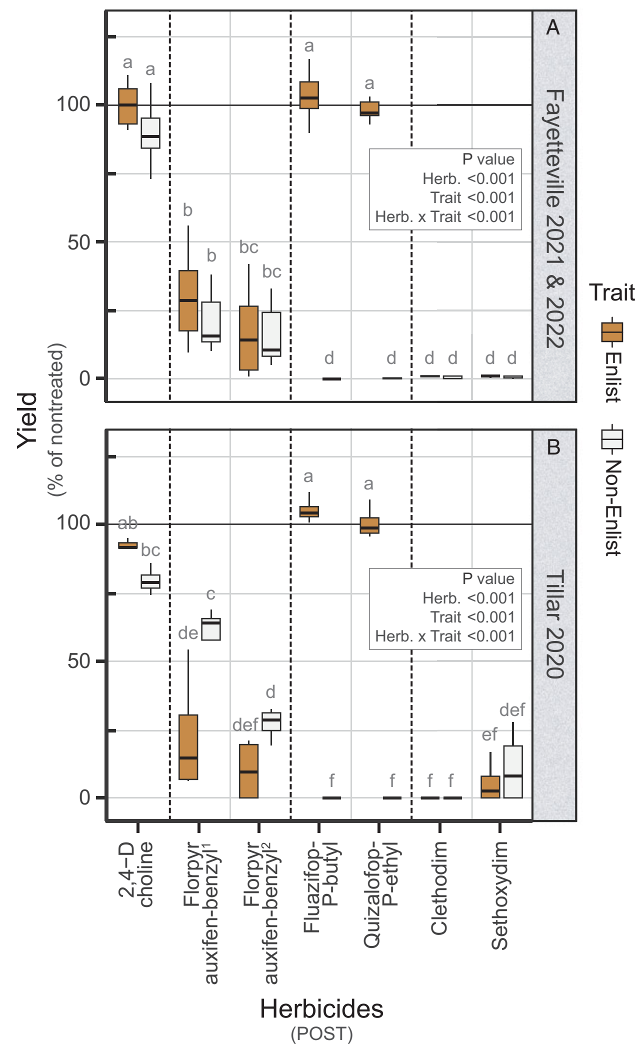

Figure 7. Yield of Enlist corn as affected by postemergence (POST) application of synthetic auxin and ACCase-inhibiting herbicides at (A) the Fayetteville site (2021 and 2022) and (B) the Tillar site (2022) in Arkansas. Yield is expressed as a percent of the nontreated control. ANOVA output of a generalized linear mixed model (herbicide and trait as fixed factors, and block as random factor) are shown at the right side of each plot. Means with the same letter within each plot are not significantly different ($P < 0.05$ using Tukey's honestly significant difference test). [1]1× rate; [2]2× rate. Herb. indicates herbicide.

Corn with the AAD-1 trait and conventional corn are both susceptible to DIM herbicides; indeed, their relative susceptibilities are expected to be similar, albeit not well established. This has significant ramifications for managing volunteer corn.

The initial development of corn injury with florpyrauxifen-benzyl was moderate and persisted through the final evaluation point (i.e., 6 WAA) according to the rate applied, with non-Enlist corn generally exhibiting greater injury compared to Enlist corn (data not shown). Plant height measurements generally corroborated well with the injury assessments, indicating that the injury was at least partially comprised of crop stunting. At the Tillar location in 2020, corn responded differently to florpyrauxifen-benzyl than at other sites, resulting in a herbicide-by-trait-by-site interaction that was otherwise fairly consistent. In contrast to the greater injury or height reduction of non-Enlist corn 2 wk following application of florpyrauxifen-benzyl, grain yield at this site was greater for non-Enlist corn compared to Enlist corn. This result is noteworthy since the potential use of this rice herbicide, albeit at lower rates, is being evaluated in corn by our research group.

POST-applied FOP herbicides had no effect on Enlist corn yield, whereas DIM herbicides reduced yield by at least 90% in both hybrids. Similar results have been shown with respect to volunteer Enlist corn control from earlier field studies in Ontario (Soltani et al. 2015), and Nebraska (Striegel et al. 2020). The use rates of most herbicides in these studies were lower than rates used in this study. Crop yield was similar between Enlist and non-Enlist corn with the applied rate of 2,4-D choline; however, Enlist corn is expected to tolerate much higher than the labeled rate of 2,4-D than non-Enlist corn (Ruen et al. 2017), whereas non-Enlist corn would be at risk of developing typical synthetic auxin injury with the labeled rate (Wright et al. 2010).

Practical Implications

With direct comparisons to non-Enlist corn, this two-site, 2-yr field study evaluated Enlist corn response to both PRE- and

POST-applied synthetic auxin and ACCase-inhibiting herbicides that are relevant in mid-southern crop production systems. Results from this study indicate that transient injury is possible for Enlist corn even from those labeled herbicides, particularly when applied POST. Florpyrauxifen-benzyl, which is structurally different from 2,4-D and has a distinct site of action, initially caused a mild injury (typically <50%) but resulted in serious yield loss in both Enlist and non-Enlist corn, with instances of greater yield loss in Enlist corn than in non-Enlist corn. These results provide baseline information on outcomes from the prospective, both intended and accidental, exposure of Enlist corn to commonly used synthetic auxins and ACCase-inhibiting herbicides in mid-South agriculture. The findings of this study can be used to guide future research and provide recommendations to growers.

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