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Auxin-based herbicide program for weed control in auxin resistant soybean

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Abstract

Soybean [*Glycine max* (L.) Merr.] cultivars resistant to synthetic auxin herbicides have provided another mode of action for the postemergence broadleaf weed control. This field study was conducted at three South Dakota locations [Northeast, NERF; east-central, ARF; and Southeast, SERF) in 2019 and two locations (ARF and SERF) in 2020. The Enlist E3 and Roundup Ready 2 Xtend cultivars were planted at three dates (early, mid-, and late season) to examine weed control, agronomic characteristics, nodulation, and yield. Preemergence (PRE) treatment was flumioxazin + metribuzin + S-metolachlor + glyphosate + pendimethalin. Two postemergence (POST) treatments, based on cultivar, were compared with PRE-only. The PRE-only treatment had numerous grasses {including green foxtail [*Setaria viridis* (L.) P. Beauv.] and yellow foxtail [*S. pumila* (Poir.) Roem. & Schult.], volunteer corn (*Zea mays* L.), barnyard grass [*Echinochola crus-galli* (L.) Beauv.], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], woolly cupgrass [*Eriochloa villosa* (Thunb.) Kunth]} and broadleaf weeds (including redroot pigweed [*Amaranthus retroflexus* L.], common lambsquarters [*Chenopodium album* L.], waterhemp [*Amaranthus rudis* Sauer]) with high density and biomass. POST treatments controlled most of the broadleaf species, although some grasses remained. Yields were similar within a location and year, although differences occurred among planting dates. In 2019, planting date did not influence final yield at ARF (average yield 3,084 kg ha^{-1}). Yield was greatest for the early (NERF) and mid-planting dates (NERF and SERF) compared with late-season planting. In 2020, dry conditions occurred, and yields at ARF and SERF were lowest for the late-season plantings (ranging from 37 to 73% lower depending on cultivar) compared with the early season planting. In 2020, dicamba + glyphosate

Abbreviations: ARF, Aurora Research Farm; E3, Enlist E3 soybean cultivar; NERF, Northeast Research Farm; PD1, earliest planting date; PD2, mid-season planting date; PD3, late-season planting date; POST, postemergence herbicide application; PRE, preemergence herbicide application; SERF, Southeast Research Farm; Xtend, Roundup Ready 2 Xtend soybean cultivar.

treatment of the Xtend cultivar had 10% (ARF) and 20% (SERF) greater yield than the acifluorfen + clethodim treatment.

1 | INTRODUCTION

Synthetic auxin herbicides have been used for broadleaf weed control in grass grain crops and pasturelands since the 1950s (Busi et al., 2018). Conventional soybean [*Glycine max* (L.) Merr.] is extremely sensitive to auxin injury, from tank contamination, drift, or both (Behrens & Lueschen, 1979; Egan & Mortensen, 2012; Egan et al., 2014; Jones et al., 2019; Sall et al., 2020; Sciumbato et al., 2004; Soltani et al., 2020; Striegel et al., 2020). Depending on soybean growth stage at the time of injury and the herbicide concentration, symptoms vary from cosmetic, with leaf cupping and strapping, and stem epinasty, to complete crop destruction (Andersen et al., 2004). In addition, if conventional soybean is injured by synthetic auxin herbicides, early foliar fertilizer N applications can further reduce yield (Van de Stroet et al., 2019). Several POST broadleaf herbicide modes of action used in soybean include acetolactate synthase (ALS) inhibitors, inhibitor of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), inhibitor of glutamine synthetase, and inhibitors of protoporphyrinogen oxidase (PPO). Weeds that are resistant to one or several of these modes of action have become a threat in many soybean fields (Clay, 2021; Heap, 2022). The introduction of auxin herbicide-tolerant soybean cultivars allows for the use of 2,4-dichlorophenoxyacetic acid (2,4-D) or 3,6-dichloro-2-methoxybenzoic acid (dicamba). This expands the possibilities for another POST broadleaf control herbicide family (although a few auxin-resistant weed biotypes have been reported [Busi et al., 2018; Heap, 2022]) and effectively eliminates sensitivity issues seen in conventional soybean.

Soybean generally is not fertilized with nitrogen (N). Rather, the plant relies on N_2 fixed from symbiosis with N-fixing bacteria (*Bradyrhizobium japonicum*) in root nodules to provide enough N for plant health and grain production. It is estimated that a 4,700 kg ha^{-1} grain crop needs about 270 kg N ha^{-1}, and that soybean has a maximum N_2 fixation capacity of about 340 kg ha^{-1} under ideal environmental conditions (Salvagiotti et al., 2008). Peak nodulation occurs between R2 (flowering) and R5 (pod set) (Licht, 2014; Lindermann & Ham, 1979). Soybean nodulation is inhibited by high soil N levels (Gresshoff, 1990), or stress from weed presence (Gal et al., 2015), herbicide applications (Tortosa et al., 2021), or high root temperatures (Lindermann & Ham, 1979). In addition, laboratory studies have shown that high natural auxin levels in soybean roots also reduce soybean nodulation (Turner et al., 2013). Auxin herbicides can be translocated to soybean roots after application (Linscott &McCarty, 1962; Skelton et al., 2017), however the impact of applying synthetic auxin herbicides on nodulation of synthetic auxin-tolerant soybean has not been examined in field settings. This 5 site-year study examined weed management, soybean growth, nodulation, yield, and grain protein of two synthetic auxin-tolerant soybean cultivars {Enlist E3 (Stine Seed Company) , resistant to 2,4-D, glufosinate [2-amino-4-[hydroxy(methyl)phosphoryl]butanoic acid] and glyphosate [N-(phosphonomethyl)glycine], and Roundup Ready 2 Xtend (Asgrow) resistant to dicamba and glyphosate} (hereafter E3 and Xtend, respectively).

2 | MATERIALS AND METHODS

2.1 | Field locations

Field studies were conducted at three South Dakota locations in 2019, a Northeast location (South Shore, Northeast Research Farm, NERF hereafter) (45°06′ N, 97°06′ W), an east-central location (Aurora Research Farm, ARF hereafter) (44˚18′ N, 96°40′ W) near Brookings, and a Southeast location (Southeast Research Farm, SERF hereafter) (43° 02′ N, 96° 54′ W) near Beresford. The study was repeated in 2020 at ARF and SERF. The soil types at the experimental locations were Brookings clay loam with 0–2% slope (fine-silty, superactive, frigid Cumulic Hapludoll) at NERF, Brandt silty clay loam with 0–2% slope (fine-silty, mixed, superactive, frigid Calcic Haplodoll) at ARF, and Egan silty clay with 0–2% slope (fine-silty, mixed, superactive, mesic Udic Hapludoll) at the SERF.

The Köppen climate classification subtypes for the study locations were “Dfb” (warm humid continental climate) for NERF and ARF and “Dfa” (hot summer continental climate) for SERF (https://www.weather-base.com/search/search.php3?query=south+dakota). Compared with the 30-yr average growing degree days (GDD) (base 10 ˚C) from the earliest planting date to harvest (Table 1), GDD were 14% lower than normal for NERF and 8% lower than normal for ARF in 2019 (30-yr average 1,383 and 1,388, respectively) and similar to the 30-yr average at ARF in 2020 and SERF (30-yr average 1,560) in 2019 and 2020. Total seasonal rainfall differed between years. The 2019 season was wet in each location with rainfall totals 50 (SERF) to 170 (ARF) mm above the 30-yr average (about 410 mm NERF; 430 mm ARF; and 470 mm SERF). The 2020 season was dry, with rainfall 25 and 53% below the 30-yr average at the ARF and SERF locations, respectively.

Core Ideas

- Auxin-resistant soybean cultivars planted at three locations and dates had similar yields within a location.
- Preemergence-only treatments had several grass and broadleaf weeds and high weed biomass.
- Pretreatments followed by postemergence treatments had fewer weed species and lower weed biomass.
- Soybean nodulation was influenced by planting date and weed presence but not by herbicide treatment.

2.1.1 | Land preparation and planting

At NERF and ARF, the entire plot area of the fields were disked to a depth of about 10 cm about a week before the first planting and then field cultivated on the date of the first planting, whereas SERF was under no-till system. The previous crop at all locations was corn (*Zea mays* L.). E3 and Xtend soybean crops were sown at 350,000 seeds ha^{-1} to a depth of 2.5 cm on 0.76-m row spacing at three spring planting dates (early-PD1, mid-PD2, and late-PD3) (Table 1) at each location. PD1 occurred as soon as planting could occur when spring soil temperatures at 5 cm were at least 10 ˚C. PD2 was the typical target planting date for soybean in South Dakota, late May to early June. PD3 was targeted for mid-June. Due to spring rains in 2019, these dates were adjusted to meet environmental conditions (Table 1). Relative maturity groups (MG) (as designated by the company seed source) differed by location, with 1.0–1.1 MG (short maturity) planted at NERF; 1.3–1.7 MG (mid-maturity rating) planted at ARF; and 2.0 MG (longer maturity) planted at SERF (Table 1). E3 seed was untreated, whereas Xtend seeds were pretreated with the labelled rate of fungicide/insecticide combination of metalaxyl [methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate], fluxapyroxad [3-(difluoromethyl)−1-methyl-N-(3′,4′,5′-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide], and pyraclostrobin [methyl N-[2-[[1-(4-chlorophenyl)pyrazol-3-yl]oxymethyl]phenyl]-N-methoxycarbamate] (Acceleron, BASF). Each chemical was applied at a target rate of 0.02 mg a.i. per seed.

A preemergence (PRE) herbicide tank mix {flumioxazin [N-(7-fluoro-3,4-dihydro-3-oxo-4-prop-2-ynyl-2H-1,4-benzoaxin-6-yl)cyclohex-1-ene-1,2-dicarboxamide]} 420 g a.i. ha^{-1} + metribuzin (4-amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one) 560 g a.i. ha^{-1} + glyphosate 340 g a.e. ha^{-1} + S-metolachlor {2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)−1-methoxypropan-2-yl]acetamide]} 120 g a.i. ha^{-1} + pendimethalin (3,4-dimethyl-2,6-dinitro-N-pentan-3-ylaniline) 290 g a.i. ha^{-1} + ammonium sulfate (3 kg ha^{-1}) was applied to all plots within about 6 d of each planting date to burndown any emerged weeds and provide residual weed control, especially for grass weeds. The exception for the PRE application timing was in 2020 at SERF when the PRE treatment for all planting dates was applied on 11 May, 4 (PD1)–27 d (PD3) prior to planting, due to COVID travel restrictions.

For each soybean cultivar, a PRE-only herbicide treatment (no POST application) was used to evaluate weed problems if only a preemergence herbicide was applied. The POST herbicide treatments (Table 2) were applied to the other plots based on soybean cultivar and were applied on the same calendar date regardless of planting date. PD1 plants (earliest planting date) ranged from V3 to V5 stage of growth, whereas PD3 plants (latest planting date) were between the VC and V2 growth stage (Table 2). The two POST herbicide treatments applied to the E3 soybean were the choline salt of 2,4-D 0.54 kg a.e. ha^{-1} + clethodim {2-[(E)-N-[(E)−3-chloroprop-2-enoxy]-C-ethylcarbonimidoyl]−5-

TABLE 1 Soybean cultivars, relative maturity groups, planting dates and growing degree days (GDD) from planting to harvest at Northeast Research Farm (NERF), Aurora Research Farm (ARF), and Southeast Research Farm (SERF) in South Dakota in 2019 and 2020 growing seasons

					2019			2020		
Location	Soybean cultivar	Maturity group	Days to maturity	GDD	PD1	PD2	PD3	PD1	PD2	PD2
NERF	Enlist E3 (Stine[a] 11EC20)	1.1	≤120		15 May	30 May	15 June	–	–	–
	Roundup Ready 2 Xtend (Asgrow[b] 10 × 9)	1.0		GDD[c]	1,178	1,136	1,008			
ARF	Enlist E3 (Stine 13EA12)	1.3	≤127		15 May	2 June	19 June	20 May	3 June	16 June
	Roundup Ready 2 Xtend (Asgrow 17 × 8)	1.7		GDD	1,279	1,198	1,047	1,420	1,314	1,166
SERF	Enlist E3 (Stine 22EB23)	2.0	≤137		7 May	5 June	19 June	15 May	29 May	12 June
	Roundup Ready 2 Xtend (Asgrow 20 × 7)	2.0		GDD	1,535	1,378	1,236	1,552	1,458	1,292

[a]Stine Seed Co.

[b]DEKALB Asgrow Seed, Bayer CropScience.

[c]GDD (growing degree days) were calculated using base 10˚C growing degree days from each respective planting date to harvest. Thirty-year (1981–2010) average GDD from first planting date to harvest are 1,383 for NERF; 1,388 for ARF; and 1,560 for SERF.

TABLE 2 Applications dates of Enlist E3 and Roundup Ready 2 Xtend treatments and soybean growth stage by planting date (PD) in 2019 at all three locations and 2020 at Aurora Research Farm (ARF) and Southeast Research Farm (SERF)

		Enlist E3 treatments[a]		Roundup Ready 2 Xtend treatments[b]			
Location	PD	POST herbicide treatment date	Soybean growthstage	Post dicamba treatment date	Soybean growth stage	Post acifluorfen treatment	Soybean growth stage
2019							
NERF	15 May	15 July	V5	27 June	V3	15 July	V5
	30 May		V3		V1		V3
	15 June		V2		VC		V2
ARF	15 May	15 July	V5	27 June	V4	15 July	V5
	2 June		V3		V1		V3
	19 June		V2		VC		V2
SERF	7 May	16 July	V5	25 June	V3	16 July	V5
	5 June		V3		V1		V3
	19 June		V2		VC		V2
2020							
ARF	20 May	19 July	V5	24 June	V3	16 July	V5
	3 June		V3		V1		V3
	16 June		V2		VE		V2
SERF	15 May	22 July	V5	24 June	V2/V3	22 July	V5
	29 May		V3		V1		V3
	12 June		V2		VE		V2

[a]Enlist E3 herbicide treatments were choline salt of 2,4-D + clethodim (Enlist One + Select Max) at 0.54 + 0.13 kg a.e. or a.i. ha^{-1} or 2,4-D + glufosinate (Enlist One + Liberty 280 SL) at 0.54 + 0.30 kg a.e. ha^{-1}. Enlist One – Corteva Agriscience; Select Max and Liberty, Bayer CropScience.
[b]Xtend herbicide treatments were dicamba + glyphosate (XtendiMax + PowerMAX) at 0.28 + 0.34 kg a.e. ha^{-1} or acifluorfen + clethodim (Acifin 2L + Select Max) at 0.18 + 0.13 kg a.e. or a.i. ha^{-1}. XtendiMax and PowerMAX, Bayer CropScience; Acifin, Summit Agro.

(2-ethylsulfanylpropyl)−3-hydroxycyclohex-2-en-1-one} 0.13 kg a.i. ha^{-1} or 2,4-D + glufosinate 0.30 kg a.e. ha^{-1}. The two herbicide treatments applied to the Xtend soybean were diglycolamine salt of dicamba 0.28 kg a.e. ha^{-1} + glyphosate 0.34 kg a.e. ha^{-1} or acifluorfen [propyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]−2-nitrobenzoate] 0.18 kg a.i. ha^{-1} + clethodim 0.13 kg a.i. ha^{-1}. Spray additives of Class Act Ridion (WinField United, Land O'Lakes Inc.) at 1.2 L ha^{-1} and the non-ionic surfactant Chemsurf 90 at 3.0 L ha^{-1} (WinField United, Land O'Lakes Inc.) were also added to each herbicide mix.

Herbicides were applied with a CO_2-pressurized bicycle-type sprayer calibrated to deliver 187 L ha^{-1} at a ground speed of 4.5 km h^{-1}. The nozzles were set at 46 cm above the crop. For acifluorfen treatments, XR TeeJet (TeeJet Technologies) (XR8002) extended range flat spray were used at 276 kPA, which produced a fine droplet size (145–225 microns). For the 2,4-D and dicamba treatments, specific drift-reducing nozzles operating in specific pressure ranges were used to comply with U.S. Federal laws. For the 2,4-D treatments, AI TeeJet (AI11002) air-induction flat spray tips were used with a spray pressure of 276 kPA, which produced an extremely coarse droplet size (501–650 microns). For dicamba treatments, Turbo TeeJet Induction (TTI11003) flat spray tips were used at a spray pressure of 207 kPa, which produced an ultra-coarse spray droplet (>650 microns).

2.1.2 | Data collection

In three 0.1 m^2 quadrats per plot, weeds were counted and cut about 2 cm above the soil surface between the middle soybean rows 6 wk after POST herbicide treatment applications (WAA). Plants were oven dried at 60 ˚C for 5 d, and biomass quantified.

At R5 stage of soybean growth (beginning of seed development), the greenness index was measured from four soybean plants per plot using the Chlorophyll Meter SPAD-502Plus [Konica Minolta, Inc.] with the average value recorded. The sampled plants were cut near the soil surface, dried, and biomass quantified. Root samples beneath two of the measured plants were collected in soil cores (11-cm diam. to 7.6-cm depth) using a standard golf hole cutter (Stegmann Golf International) centered over the stem. Cores were stored at 3 ˚C until root nodule evaluation, within 5 d after soil sampling.

In the laboratory, soil was removed from soybean roots by soaking each core individually in a pail containing water and soap (Liquinox, Alconox). After soil dispersion, roots were collected and nodules remaining on the roots were counted. In addition, the soil slurry was passed through a series of sieves with detached nodules collected and counted to provide a count of total nodules per core. Nodules were cut and red/pink nodules were considered active, whereas green, white, and black nodules indicated inactive, immature, and dead nodules, respectively. Nodule numbers were averaged between the two cores per plot and reported on a 500-cm^3 soil volume.

At crop physiological maturity (R7 – when one pod on main stem of about 80% of the plants within a location reached mature pod color), the aboveground weed biomass was collected and quantified as described above. After removing the plot edges, the middle two rows of the plots were harvested at R8 (full maturity) with a small plot combine and seeds dried at 60 ˚C for 7 d. After debris was removed using blowers and screens, cleaned grain was weighed and yield (13% moisture basis) per hecatare was calculated per plot. Seed oil and protein for a 500-g subsample were measured using near-infrared techniques using a calibrated FOSS Intratec 1229 Whole Grain Analyzer (Foss Tecator AB). One-hundred seeds were counted and weighed to determine the 100-seed weight of the sample.

2.1.3 | Experimental design and statistical analysis

Treatments at all locations were arranged by soybean cultivar (E3 or Xtend) in a split plot design with four replications. Planting dates (early season–PD1, midseason–PD2, or late season–PD3) were the main plot whereas herbicide treatments (two appropriate POST treatments for each soybean cultivar and a PRE-only treatment for each cultivar) were the subplots. Subplots were four rows wide by 9 m long. An untreated buffer of 15 m was established between the two cultivars.

Due to differences in soybean maturity group among locations, weed species observed, and environmental conditions between years, data obtained from each cultivar/maturity group, location, and year were analyzed independently using the R – statistical software package (http://www.r-project.org). Herbicide and planting dates parameters were fixed effects, whereas blocks were random. The fixed effects of herbicide and planting date were tested using Type II statistics. Square root transformation of weed density data was performed to improve homogeneity of variance. All data were subjected to ANOVA using the linear mixed effect procedure in R. Treatment means were separated using $P \leq .10$ (due to high sample variation) using the Fisher's Least Significant Difference (LSD) (Steel et al., 1997) and, when appropriate, back-transformed data are reported.

3 | RESULTS

3.1 | Weed management

3.1.1 | Weed species

The PRE-only treatment had the greatest weed species diversity based on visual observations for all 5 site-years at 6 wk after the POST application (data not shown) and at harvest (Table 3). Grass and broadleaf species differed somewhat by location, but were similar within location, between cultivars and years.

Grasses observed at all three locations in the PRE-only treatment for most of the planting dates included green and yellow foxtail [*Setaria viridis* (L.) P. Beauv. and *S. pumila* (Poir.) Roem. & Schult., respectively], and barnyard grass [*Echinochola crus-galli* (L.) Beauv.]. Woolly cupgrass (*Eriochloa villosa* (Thunb.) Kunth] was noted at NERF and ARF. Fall panicum (*Panicum dichotomiflorum* Michx.), field sandbur [*Cenchrus longispinus* (Hack.) Fern.], and foxtail barley (*Hordeum jubatum* L.) were only observed at SERF for PD2 and PD3.

Several broadleaf species were observed in the PRE-only treatment. Dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers), wild buckwheat (*Polygonum convolvulus* L.), and redroot pigweed (*Amaranthus retroflexus* L.) were noted at all locations at one or more planting dates (Table 3). Common waterhemp (*Amaranthus rudis* Sauer) at ARF and SERF, and marestail [*Conyza canadensis* (L.) Cronq.] at SERF were observed. These species have glyphosate-resistant biotypes reported in South Dakota (Heap, 2022).

POST treatments generally had fewer grass and broadleaf weeds than the PRE-only treatment and most were not present at all PDs (Table 3). The POST treatments of 2,4-D + clethodim (E3) and acifluorfen + clethodim (Xtend) had the greatest grass species diversity at SERF especially in the PD2 planting. Across locations, barnyard grass and green foxtail were the most common grasses noted with observations in 10 and 6 of the 12 POST-treatments, respectively.

Eight of the 12 POST treatments had no broadleaf weeds present. Common lambquarters was the weed observed in POST treatments of 2,4-D + clethodim (E3) and acifluorfen + clethodim (Xtend) at NERF in the PD2 planting. Common waterhemp and marestail were noted in the acifluorfen + clethodim (Xtend) at SERF in the PD3 planting.

3.1.2 | Weed biomass

End-of-season weed biomass was not influenced by planting date ($P > .1$) within a year/location and therefore data were combined. Herbicide treatment within a year/location and

TABLE 3 Weeds present in herbicide treatments by location (NERF – Northeast Research Farm, South Shore, SD 2019; ARF- Aurora Research Farm, Aurora, SD 2019 and 2020; SERF- Southeast Research Farm, Beresford, SD 2019 and 2020) at the end of season sampling. All cultivars received the same pretreatment, whereas the 2,4-D based POST treatments were only applied to the Enlist E3 soybean cultivar, and the acifluorfen and dicamba based POST treatments were applied to the Roundup Ready 2 Xtend soybean cultivar. Weed species in the PRE-only treatment were observed in both the Enlist E3 and Roundup Ready 2 Xtend soybean cultivars. Presence of weed species observed by planting date treatment (PD) provided where A represents presence in all three PDs and 1, 2, and 3 represent presence in early-season, mid-season, and late-season PDs, respectively

		Location					
Treatment	**Plant type**	**NERF**	**PD**	**ARF**	**PD**	**SERF**	**PD**
PRE-only	grass	barnyard grass [*Echinochola crus-galli* (L.) Beauv.]	A	Barnyard grass	A	barnyard grass	1,3
		green foxtail [*Setaria viridis* (L.) P. Beauv.]	A	green foxtail	2,3	fall panicum (*Panicum dichotomiflorum* Michx.)	2,3
		large crabgrass [*Digitaria sanguinalis* (L.) Scop.]	A	quackgrass [*Elytrigia repens* (L.)Desv. Ex B.D. Jackson]	2,3	field sandbur [*Cenchrus longispinus* (Hack.) Fern.]	2,3
		volunteer wheat (*Triticum aestivum* L.)	A	volunteer corn (*Zea mays* L.)	1	foxtail barley (*Hordeum jubatum* L.)	2,3
		woolly cupgrass [*Eriochloa villosa* (Thunb.) Kunth]	A	woolly cupgrass	2,3	green foxtail	2,3
		yellow foxtail [*S. pumila* (Poir.) Roem. & Schult.]	A	yellow foxtail	2,3	large crabgrass	2,3
						volunteer corn	1
						yellow foxtail	2,3
	broadleaf	common lambsquarters (*Chenopodium album* L.)	A	common lambquarters	A	common waterhemp	A
		common purslane (*Portulaca oleracea* L.)	A	common waterhemp (*Amaranthus rudis* Sauer)	3	Dandelion	3
		dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers)	A	dandelion	1,3	marestail [*Conyza canadensis* (L.) Cronq.]	A
		prostrate pigweed (*Amaranthus blitoides* S. Wats.)	A	lady's thumb (*Polygonum persicaria* L.)	2,3	redroot pigweed	1,3
		redroot pigweed (*Amaranthus retroflexus* L.)	A	redroot pigweed (*Amaranthus retroflexus* L.)	3	wild buckwheat	3
		wild buckwheat (*Polygonum convolvulus* L.)	A	velvetleaf (*Abutilon theophrasti* Medik.)	A		
				wild buckwheat	A		
				wild four o'clock (*Mirabilis nyctaginea* (Michx.) MacMill.]	3		
Enlist cultivar POST treatments							
2,4-D + clethodim	grass	barnyard grass	1	green foxtail	2	barnyard grass	2,3
		volunteer wheat	1,2	volunteer corn	2,3	green foxtail	1,2
						large crabgrass	2,3
						volunteer corn	1
	broadleaf	common lambsquarters	2	none		none	

(Continues)

TABLE 3 (Continued)

Treatment	Plant type	Location NERF	PD	ARF	PD	SERF	PD
2,4-D + glufosinate	grass	barnyard grass	3	green foxtail	1	barnyard grass	1
		volunteer wheat	3	volunteer corn	1,2	large crabgrass	1,3
	broadleaf	none		none		none	
Xtend cultivar POST treatments							
Acifluorfen + clethodim	grass	barnyard grass	3	green foxtail	2,3	Barnyard grass	2
		green foxtail	3	volunteer corn	A	fall panicum	2
		volunteer wheat	A			field sandbur	3
						large crabgrass	2
						volunteer corn	1
	broadleaf	common lambquarters	2	none		common waterhemp	3
						marestail	3
Dicamba + glyphosate	grass	barnyard grass	3	green foxtail	1	Barnyard grass	1
		volunteer wheat	3	volunteer corn	1,2	large crabgrass	1,3
	broadleaf	none		none		none	

TABLE 4 End-of-season weed biomass averaged over planting date by treatment for two soybean cultivars at three eastern South Dakota locations (Northeast Research Farm, NERF; Aurora Research Farm, ARF; Southeast Research Farm, SERF) for 2019 and 2020

	End-of-season weed biomass							
	Soybean cultivar							
	Enlist E3				Roundup Ready 2 Xtend			
Year	Treatment	NERF	ARF	SERF	Treatment	NERF	ARF	SERF
2019			g m^{-2}				g m^{-2}	
	PRE-only	220a	130a	38	PRE-only	188a	440a	172a
	2,4-D + clethodim	50b	10b	20	acifluorfen + clethodim	67b	32b	85b
	2,4-D + glufosinate	13b	50b	40	dicamba + glyphosate	23b	100b	37b
2020								
	PRE-only	na[a]	500a	700a	PRE-only	na	179a	427a
	2,4-D + clethodim	na	20b	70b	acifluorfen + clethodim	na	64b	245b
	2,4-D + glufosinate	na	38b	100b	dicamba + glyphosate	na	28b	58c

Note. Different letters within the same column by location and year indicate differences using the Fisher's test for significance at $P \leq .10$.
[a]Not applicable as NERF was not a study site in 2020.

soybean cultivar influenced weed biomass (Table 4). Weed biomass generally was greatest for PRE-only treatment in both soybean cultivars within a location by year, with only one of the nine treatments over years having similar biomass to POST treatments. Biomass in the PRE-only treatments (when it differed from POST treatments) ranged from 700 g m^{-2} in 2020 POST E3 at SERF to 130 g m^{-2} in 2019 E3 at ARF (Table 4). The 2,4-D + clethodim and 2,4-D + glufosinate treatments in the E3 soybean reduced weed biomass at NERF (2019), ARF (both years), and SERF (2020) with weed biomass averaged over planting date, ranging from 10 to 100 g m^{-2}.

In the Xtend soybean, both the acifluorfen + clethodim and the dicamba + glyphosate treatments had less weed biomass than the PRE-only treatments. In 2020 at SERF, the acifluorfen + clethodim treatment had almost five times greater

TABLE 5 Soybean plant biomass, total and active (pink/red coloration) nodule numbers, and yield for Enlist E3 soybean at three eastern SD (Northeast Research Farm, NERF; Aurora Research Farm, ARF; Southeast Research Farm, SERF) locations in 2019 and two SD locations, ARF and SERF in 2020. Plant biomass and nodule numbers obtained from samples collected at R5. Yield (13% moisture) was calculated based on sampling at soybean maturity. Averages are provided when values were similar across planting dates and herbicide treatments

2019			Nodule no.		
Year ocation	Planting date	Soybean biomass	Total	Active	Yield
		g plant^{-1}	——no. 500 cm^{-3}——		kg ha^{-1}
2019					
NERF					
	PD1				3,591a
	PD2				3,598a
	PD3				3,147b
	Average	25	40	11	
ARF	Average	17	68	11	3,084
SERF					
	PD1			9 b	
	PD2			20 a	
	PD3			20 a	
	Average	16	33		3,200
2020					
ARF					
	PD1	17a	66a	50a	3,207a
	PD2	17a	59a	40b	2,797a
	PD3	15b	43b	25c	1,632b
SERF					
	PD1	34a	49a	33a	2,770a
	PD2	28b	44a	25ab	2,232b
	PD3	21c	30b	17b	1,728c

Note. Different letters within the same column by location and year indicate differences using the Fisher's test for significance at $P \leq .10$.

weed biomass (245 g m^{-2}) than the dicamba + glyphosate (58 g m^{-2}) treatment. The difference in control between these POST-treatments was most likely due to the late application of acifluorfen (22 July) to large weeds that provided less control compared with the month earlier treatment of dicamba (24 June) (Table 2).

3.2 | In-season soybean parameters

3.2.1 | Soybean plant biomass – Enlist

In 2019 for the E3 cultivar, planting date and herbicide treatment did not influence soybean biomass at R5 within a location (Table 5). The average E3 soybean biomass by location averaged 25 g plant^{-1} (NERF), 17 g plant^{-1} (ARF), and 16 g plant^{-1} (SERF) (Table 5).

In 2020, planting date, but not herbicide treatment, impacted soybean biomass at R5 at ARF ($P = .05$) and SERF ($P \leq .01$) (Table 5). Delayed planting from 20 May (PD1) to 16 June (PD3) at ARF resulted in 13% biomass reduction at R5 (Table 5), with an average loss of 0.1 g d^{-1}. At SERF, delayed planting from 15 May (PD1) to 12 June (PD3) resulted in 38% biomass reduction, with an average loss of 0.45 g d^{-1}.

3.2.2 | Soybean plant biomass - Xtend

In 2019 at NERF, an interaction between planting date and herbicide treatment was observed for soybean biomass in the Xtend cultivar (Table 6). Dicamba + glyphosate at PD1 and PD2 had the greatest biomass (30 g plant^{-1}), acifluorfen + clethodim at PD3 had the least biomass (21 g plant^{-1}), whereas the average over the other herbicide treatments and planting dates averaged 25 g plant^{-1} (data not shown). At SERF, planting date, but not herbicide treatment, impacted plant biomass with PD3 having a greater biomass (17 g plant^{-1}) than PD2 (10 g plant^{-1}). This may have been due

TABLE 6 Soybean plant biomass, total and active (red/pink coloration) nodule numbers, and yield for Roundup Ready 2 Xtend soybean at two eastern South Dakota locations, Aurora Research Farm (ARF) and Southeast Research Farm (SERF) in 2019 and 2020. Plant biomass and nodule numbers obtained from samples collected at R5 and yield (13% moisture) was sampled at soybean maturity

	ARF					SERF			
		Nodule no.				Nodule no.			
Year/treatment	Soybean biomass	Total	Active	Yield	Planting date	Soybean biomass	Total	Active	Yield
		g plant^{-1}	——no. 500 cm^{-3}——		kg ha^{-1}	g plant^{-1}	——no. 500 cm^{-3}——		kg ha^{-1}
2019									
Herbicide treatment									
Dicamba + glyphosate		85a	81a		PD1	14b			3,679b
Acifluorfen + clethodim		56b	48b		PD2	10c			4,250a
PRE-only		69ab	62ab		PD3	17a			3,437b
Average[a]	22			3,006			23	5	
2020									
Planting date									
PD1	15a	59a	42a	3,093a		22 a	50a	33a	2,688a
PD2	15a	52a	36a	2,905b		22 a	44ab	25ab	2,285b
PD3	12b	33b	16b	894c		11 b	34b	19b	1,827c
Herbicide treatment									
PRE-only	13b	43b	33a			–	42a	25ab	2,050b
Acifluorfen + Clethodim	12b	40b	27b			–	35b	21b	2,095b
Dicamba + Glyphosate	15a	52a	34a			–	40ab	31a	2,650a
Average[a]				2,298					

Note. Different letters within the same column by location and year indicate differences using the Fisher's test for significance at $P \leq .10$.

[a]Values are averaged over planting date and herbicide treatment if no main effect or interactions were observed.

to the wet conditions in 2019 that occurred prior to planting PD3, which contributed to unfavorable growth and establishment conditions for the PD2 cohort but provided enough soil water for the PD3 planted soybean.

In 2020, planting date impacted Xtend soybean biomass (Table 6). PD1 and PD2 at both ARF and SERF had greater average biomass than PD3 (20% loss from PD1 to PD3 at ARF and 50% loss at SERF). In addition, at the ARF site only, herbicide treatment impacted soybean biomass, with soybean in the PRE-only and acifluorfen-based POST treatment having less biomass than soybean in the dicamba-based POST treatment.

3.2.3 | Soybean greenness index (SPAD)

The SPAD meter reading is a nondestructive measurement of leaf chlorophyll concentrations which is correlated with leaf N (Wood et al., 1993; Xiong et al., 2015). SPAD values for E3 soybean at R5 averaged about 41 among all treatments at NERF (2019) and ARF (2019 and 2020) locations (data not shown). However, at SERF, SPAD values for the PRE-only E3 soybean averaged 27 (relatively low, Xiong et al., 2015), whereas the 2,4-D treatments averaged 38. Low SPAD values obtained from the PRE-only treatment were probably due to weed stress. In 2020, SPAD readings for Xtend soybean averaged about 40.8 at ARF and 39.5 at SERF and were not impacted by planting date, herbicide treatment, or the interaction ($P > .05$).

3.2.4 | Nodule number

POST 2,4-D treatments on E3 soybean had little impact on either total or active nodules in either year at any location (Table 5). In 2019, average total nodule numbers per 500 cm^3 of soil for the E3 cultivar were 40, 68, and 33 at NERF, ARF, and SERF, respectively (Table 5). Active nodules were 50% of the total nodules at ARF and 25% at NERF. At SERF, active nodules were fewer for PD1 (average nine per core) compared with those found in PD2 and PD3 (average 20 per core). At NERF, total nodules in the 2,4-D treatments averaged 57, which were greater than the PRE-only treatment (average 27). In 2020, total nodules averaged 55 (60% active) at ARF and 41 (60% active) at SERF (Table 5). PD1 at both ARF and SERF had greater total and active nodule numbers than PD3.

Delaying planting at ARF from 20 May (PD1) until 16 June (PD3) resulted in 50% reduction in active nodule numbers.

In 2019, Xtend total nodule numbers at NERF, ARF, and SERF averaged 38, 72, and 23 per 500 cm^3 soil, respectively (Table 6). Only the herbicide treatment at ARF influenced total nodules. Xtend soybean with dicamba applications had 80 total nodules, whereas PRE-only had 68 total, and acifluorfen averaged 56. Nearly 100% of the nodules were active regardless of herbicide treatment.

In 2020, average total nodule numbers at ARF and SERF sites were 48 and 42 nodules per 500 cm^3 soil, respectively. Although no interaction was found between planting date and herbicide treatment, planting date influenced total and active nodule numbers. Delaying planting from mid-May to mid-June decreased total nodule number by about 38% at each location with about 62 and 43% of the total being active at ARF and SERF, respectively. Similar to 2019, acifluorfen treatment generally had fewer total and active nodules than the dicamba + glyphosate treatment (Table 6).

3.3 | Soybean yield and seed parameters

Grain yield of E3 soybean in 2019 averaged 3,443, 3,080, and 3,200 kg ha^{-1} at NERF, ARF, and SERF, respectively (Table 5). At NERF, yield was influenced by planting date, with yield about 12% lower for PD3 (3,147 kg ha^{-1}) compared with yields from PD 1 and 2 (average 3,594 kg ha^{-1}). At ARF and SERF in 2019, yield was not influenced by planting date nor herbicide treatment. In 2020, average yields at ARF and SERF were less than the 2019 yields, and planting date impacted yield. Late-planting yields were reduced by 38 and 49% at ARF and SERF, respectively, compared with the PD1 planting date (Table 5).

In 2019, 100-seed weight was similar at ARF and SERF and averaged 15 g and was not impacted by planting date or herbicide treatment (data not shown). At NERF, seed weight in the PRE-only treatment was less (15.3 g) compared with the 2,4-D based POST treatments (15.8 g). In 2020, planting date at both ARF and SERF influenced 100-seed weight with reduced weights for the late planting dates at each location (14.8 g for PD1 vs. 14 g for PD3).

In 2019, seed oil averaged 19% across locations, and herbicide treatments (data not shown). In 2020, PD2 and PD3 at ARF, PD2, and PD3 reduced seed oil content (average 17.8%) compared with that of PD1 seeds (average 19%). Seed protein content averaged 34% and in 2019 was not influenced by planting date, location, or herbicide treatment. In 2020, seed protein at both the ARF and SERF locations were least for the PD3/PRE-only treatment compared with other planting date/herbicide treatment combinations. However, the differences, although significant at $P < .10$, would probably not be of physiological or economic relevance.

Grain yields of Xtend soybean in 2019 averaged 3,221, 3,789, and 3,006 kg ha^{-1} at NERF, ARF, and SERF, respectively (Table 6). There was no interaction between planting date and herbicide application within a location. However, in 2020, yield was influenced by the main effects of planting date and herbicide application at ARF and SERF (Table 6). At ARF, PD1 and PD2 averaged 2,950 kg ha^{-1}, whereas PD3 had a 30% lower yield. At both locations, the PRE-only and POST-acifluorfen based treatments had the lowest yields compared with dicamba + glyphosate treatment.

The 100-seed weights in 2019 averaged 14.2, 17.8, and 18.3 g at NERF, ARF, and SERF, respectively. In 2020, seed weights at ARF and SERF were 16 and 39% less than 2019 weights. Herbicide treatment had minimal impact on the seed weight within location and year. Seed oil content averaged 19%, with about a 2 and 19% reduction by delayed planting from the mid-May to mid-June planting, in 2019 (NERF and SERF) and 2020 (ARF), respectively. Seed protein averaged 35% with differences less than 1% among herbicide and planting date either year.

4 | DISCUSSION

The 2019 and 2020 seasons presented different challenges to soybean production. The 2019 season was a very wet, with rainfall amounts exceeding the 30-yr average by 50% (https://mesonet.sdstate.edu/archive). Rains throughout April and May 2019 prevented the planting of most SD crop acres, as fields were near saturation. We were "running between the raindrops" from May through mid-June to get these studies planted and later applying the POST herbicide treatments. July rains drowned out most crop land areas in low-lying areas of eastern South Dakota resulting in low harvestable land areas and low crop yields. In 2020, low rainfall and high temperatures occurred in July at SERF, and August at ARF, which resulted in drought stress during critical soybean development periods and lowered yield potentials. Soybean plants are most sensitive to drought during flowering and early pod-fill growth stages resulting in floral abortion, reduced pod number, fewer seeds, and reduced seed size (Hall & Twidwell, 2002). In addition, moderate drought stress may reduce or stop N_2 fixation further disrupting seed development (Lenssen, 2012). Although drought stress early in seed fill can reduce the number of seeds per pod, drought stress later in development can reduce seed weight (Desclaux et al., 2000).

Planting date had a large impact on both in-season growth and end-of-season yield, with late-planted soybean (about 2 wk after the location's average planting date) having smaller in-season plants, fewer nodules, and lower yields than those planted early (7–10 d earlier than the regional normal planting date) or mid-season (yearly target date for soybean planting at each location). The early and mid-planting dates in 2019 had yields that generally were greater than the 2019 SD state average of 2,860 kg ha^{-1}, and in 2020 had close to the 2020 SD state average of 3,100 kg ha^{-1} (USDA/NASS Quick Stats).

Early sowing allows more nodes to accumulate throughout the growing season (Bastisdas et al., 2008; Licht et al., 2013; Nleya et al., 2020; Staton, 2011) and there is often a strong positive correlation between the number of soybean nodes and yield (Ball et al., 2001). Soybean nodes develop at a consistent rate (estimated at 0.27 nodes per day) regardless of weather conditions (Bastidas et al., 2008). Therefore, delayed planting reduces the duration of both vegetative and reproductive phases of crop growth. Across locations and for both study years, we observed that the late-planted soybean was shorter, had fewer nodes (authors' observations for 2019 and 2020), and had less per plant biomass compared with early planted soybean. However, sowing soybean early in South Dakota is not without perils. First, soil temperatures must be warm enough and have optimal moisture conditions to support germination, emergence, and growth of soybean. Intercontinental springs can be very cold and dry. In addition, once the plant emerges mid- and/or late-season frosts cannot be tolerated and will kill the soybean growing point. Although late planting does not experience cold spring soils or frosts, early fall frosts (early to mid-September) can injure or kill late-planted soybean, resulting in reduced or no yields.

In both of the auxin-tolerant soybean cultivars, grass weeds were present at the three study locations. Although the PRE-only treatment contained residual grass herbicides and POST-treatments had herbicides that should have controlled grasses (e.g., clethodim, glyphosate, and glufosinate), most of the POST treatments had some grass weeds present at harvest. This may have been due to antagonism between the herbicides chosen for the tank-mix. For example, auxin herbicides have been reported to reduce translocation of clethodim and glyphosate herbicides in grasses (Merritt et al., 2020). Specifically, when compared with clethodim alone, 2,4-D + clethodim reduced the control of volunteer wheat (*Triticum aestivum* L.) (Blackshaw et al., 2006) and dicamba + clethodim had poorer control of volunteer corn in dicamba-tolerant soybean (Underwood et al., 2016). Broadleaf weed control was excellent, except for waterhemp and marestail (both of which have been confirmed as glyphosate-resistant) at SERF. Previous studies have reported less control of kochia [*Bassia scoparia* (L.) A.J. Scott], which can be a major weed of concern in South Dakota fields, when dicamba was tank-mix with glyphosate, compared with dicamba alone (Flint & Barrett, 1989; Ou et al., 2018).

In our study, the tank-mix of 2,4-D + glufosinate had similar POST broadleaf and grass control as the tank-mix with clethodim. Craigmyle et al. (2013) and Frane et al. (2018) both reported that POST application of 2,4-D + glufosinate provided effective control (about 85%) of annual grasses (except large crabgrass) and broadleaf weeds, including glyphosate-resistant broadleaves, compared with 2,4-D or glufosinate applied alone.

Planting date by herbicide interactions were not found to be significant for chlorophyll values, and soybean plant biomass for either cultivar. These results are similar to Silva et al. (2021) who reported no herbicide effects of 2,4-D choline, glyphosate, or glufosinate on chlorophyll indices of E3 soybean. Albrecht et al. (2018), however, reported reductions in chlorophyll indices when higher rates (2,880 g a.e. ha^{-1}) of glyphosate were applied at V4 growth stage in Roundup Ready soybean. Plant biomass was only influenced by acifluorfen POST at ARF in 2020. Acifluorfen is known to bronze, crinkle, or cause necrotic spots on leaves due to the accumulation of tetrapyrroles (Matringe & Scalla, 1988), which may reduce growth within 2 wk after application, and subsequently delay canopy closure (Priess et al., 2020).

Because weed, soil moisture, herbicide, and temperature stresses have been reported to impact nodule number and activity, we expected that nodule numbers would differ among treatments. Soil moisture levels from high rainfall amounts just prior to R5 at NERF in 2019 reduced the number of active nodules in both soybean cultivars. Wet and/or flooded soil conditions result in decayed and rotten nodules, reducing active nodule numbers (http://msue.anr.msu.edu/news/evaluating_soybean_nodulation).

The application of auxin herbicides may increase auxin in the root (Linscott & McCarty, 1962; Skelton et al., 2017), which has been reported to decrease nodule number (Turner et al., 2013). Due to staggered planting dates in this study, auxin herbicides were applied at several soybean growth stages from VC to V5 with none of the applications impacting the number of active nodules, and nodule numbers in all treatments were above the number suggested for good N_2 fixation (Staton, 2011). In fact, Xtend soybean treated with dicamba tended to have greater total and active nodule numbers than the acifluorfen POST-treatment. The limited or no impact of synthetic auxin herbicides on nodulation was most likely due to rapid metabolism of the synthetic auxin in these tolerant soybean cultivars (Beherns et al., 2007; Skelton et al., 2017) and limited translocation to areas below the treated leaves (1–3% in 72 h) (Skelton et al., 2017). For example, in the E3 soybean, almost 100% of the 2,4-D taken up by the soybean is metabolized to the nonherbicidal dichlorophenol metabolite within 24 h after application (Skelton et al., 2017). This can be compared with results from a previous study in non-GMO soybean when 2,4-D was applied at 1/10th a labelled rate and dicamba was applied at 1/100th of a labelled rate, and 2,4-D was detected in foliage at 12 d after application and dicamba at 24 d after application (Anderson et al, 2004). In Xtend soybean, a demethylase gene encodes for dicamba monooxygenase protein that catalyzes oxidative demethylation of dicamba to 3,6-dichlorosalicyclic acid and formaldehyde (Beherns et al., 2007; Taylor et al., 2017), both of which may not impact nodulation.

In our study when higher weed densities and biomass were present, such as in the PRE-only treatments, and some of the acifluorfen + clethodim plots, decreased nodule numbers were observed. These reductions may have been due to weed stress, which has been reported to reduce nodulation (Chaniago et al., 2012; Gal et al., 2015; Tortosa et al., 2021), due to a reduction in photosynthetic energy (Francisco & Harper, 1995; Schultze & Kondorosi, 1998; Walsh, 1995) and alteration of red/far red signaling that influences molecular and physiological plant functions (Gal et al., 2015).

Early- to mid-season planting with the best adapted maturing cultivar for the location would be beneficial to obtain the highest yields, which are similar to results reported by Nleya et al. (2020). Based on this study, producers should not rely on PRE-only treatments to provide season-long control. Dicamba and 2,4-D based POST treatments provided excellent broadleaf weed control in auxin-tolerant soybean but must be properly managed due to restrictions on auxin applications. Auxin applications must be completed by the state or federal cut-off date and also within wind and rainfall restrictions. Hartzler (2017) in an Iowa scenario reported that high temperatures (≥29 ˚C), rainfall, and wind speed (<4.8 kph or >16 kph) restricted application to only a few hours within the timeframe when the weeds would also be at the optimal size for control. Therefore, producers need to be acutely aware of early-season conditions and nimble to complete the applications within the window of opportunity.

Stacking of resistance genes in these GMO soybean cultivars allows for tank-mixing of multiple herbicide chemistries with the same or different modes of action to be applied at one time, controlling both grasses and broadleaf weeds. However, some mixes reduced weed response, most likely due to antagonism. Results from our study found decreased grass weed control when grass (clethodim) or broad-spectrum (glyphosate) herbicides were applied in specific tank-mixes with auxin herbicides. Applying herbicides separately with a specified interval between applications may prevent antagonism and increase herbicide activity for optimum control of weeds.

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AUTHOR CONTRIBUTIONS

Joy Amajioyi: Formal analysis; Investigation; Methodology; Writing – original draft. Thandiwe Nleya: Writing – review & editing. Graig Reicks: Methodology; Writing – review & editing. Janet Moriles-Miller; Writing – review & editing. David E. Clay: Resources; Writing – review & editing. Sharon A Clay: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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