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Impact assessment of residual soil-applied pre-emergence herbicides on the incidence of soybean seedling diseases under field conditions

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ABSTRACT

A multi-environmental field study was conducted in 2017 and 2018 in Nebraska to investigate potential interactions between soybean seedling diseases and soil-applied residual pre-emergence (PRE) herbicides. Experiments were established from mid-May to early June in fine-textured, poorly drained soils with a history of seedling establishment problems. PRE herbicides consisted of chlorimuron-ethyl, flumioxazin, metribuzin, saflufenacil, and sulfentrazone applied at labeled rates, in addition to non-treated control. Assessments included soybean injury, seedling root lesion severity (DSI), plant height, population, biomass, and yield. Additionally, symptomatic seedling roots were sampled for fungal and oomycete organisms to expand comprehension of potential biotic associations. Greater soybean injury and reduced root biomass were observed in two distinctive environments following PPO-inhibiting PRE herbicide applications. Exceptionally in one environment, where DSI seemed ($P = 0.07$) lower for metribuzin in comparison to saflufenacil, PRE herbicides did not affect seedling root rot severity and no yield differences occurred among treatments. Community composition depicting *Fusarium*, *Phytophthora*, *Pythium,* and *Rhizoctonia* genera varied considerably across environments ($P < 0.001$) and DSI classes ($P = 0.002$), representing distinctive ecological environments under investigation. *Phytophthora* structured a large portion (>40%) of the total primary pathogenic isolates recovered in the highest DSI environment, whereas *Pythium* frequency ranged from 4.6% to 22% across all surveyed environments, and *Rhizoctonia* recovery was low (<10.3%) and sporadic. Across environments with varying DSI and soilborne pathogen composition, results indicated a lack of consistent interaction between soil-applied residual PRE herbicides and the incidence of soybean seedling diseases in optimal to delayed planting situations.

1. Introduction

Seedling diseases pose a major threat to soybean production. Annual losses are estimated at 1.3 million metric tons in North America (Allen et al., 2017; Bandara et al., 2020). A composite of soilborne pathogens is associated with seedling diseases in the United States, including *Fusarium*, *Pythium*, *Phytophthora*, and *Rhizoctonia* (Ajayi-Oyetunde and Bradley, 2017; Radmer et al., 2017; Rizvi and Yang, 1996). Symptoms include seedling damping-off, root rot, stunting, and uneven crop emergence that can additionally provide weeds a competitive advantage over the crop for the remaining of the growing season. Soybeans are most vulnerable to infection during the first days of emergence. Furthermore, edaphic and climatic conditions play a significant role in disease epidemics (Rojas-Flechas et al., 2017b; Workneh et al., 1999). Unraveling vulnerability factors associated with seedling diseases can help outline management strategies to alleviate the impact of this malady.

Soil-applied residual PRE herbicides represent a pivotal component of weed management and herbicide resistance mitigation programs in conventional and genetically modified soybeans (Norsworthy et al., 2012). Adoption of PRE herbicides into a diversified weed management strategy allows rotation of herbicide sites of action while providing early-season residual weed suppression and increasing post-emergence herbicide efficacy (Arneson et al., 2019; Jhala et al., 2017; Knezevic et al., 2019). Protoporphyrinogen oxidase (PPO)-inhibiting herbicides, including flumioxazin, saflufenacil, and sulfentrazone, have been

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increasingly adopted as part of weed management programs in soybeans in the United States (Sarangi and Jhala, 2018). For instance, between 2012 and 2020, soybean acreage treated with sulfentrazone increased from 8% to 21%. Similarly, during the same period, saflufenacil and metribuzin consumption increased by 138% and 500%, respectively (USDA, 2012, 2020). Increased reliance on PPO-inhibiting PRE herbicides is linked to their superior efficacy on troublesome weeds, including glyphosate-resistant biotypes (Krausz et al., 1998; Oliveira et al., 2017). Sarangi et al. (2017) observed a reduction in glyphosate-resistant common waterhemp (*Amaranthus rudis* L.) density from 107 to 13 plants m^{-2} upon the use of flumioxazin + chlorimuron-ethyl applied PRE followed by fomesafen + glyphosate POST compared to glyphosate applied alone. Meanwhile, sulfentrazone and flumioxazin applied PRE provided 81%–92% and 88%–98% control of kochia (*Kochia scoparia* (L.) Schrad), respectively, within 10 weeks after treatment (Hulse, 2012). Occasionally, however, PPO-inhibiting PRE herbicides cause injury to soybean, including leaf burn, desiccation, and chlorosis leading to stand reduction and yield losses (Miller et al., 2012; Zhaohu et al., 1999). A variety of factors have been suggested to enhance PPO-inhibiting herbicide injury, including applications made with an interval superior to three days from planting or during soybean emergence, soybeans cultivated in conventional tillage systems, moisture for herbicide activation, and edaphic factors (Hager, 2014; Mahoney et al., 2014; Reiling et al., 2006). Not all soybean varieties respond equally to PPO-inhibiting chemistries and tolerant lines should be considered under high-injury risk scenarios to mitigate yield losses (Taylor-Lovell et al., 2001). The risk of PRE herbicide injury is greatest when seedling emergence coincides with cool and saturated soil conditions, which is also conducive to the occurrence of certain early-season soilborne diseases in soybeans (Kirkpatrick et al., 2006; Serrano and Robertson, 2018).

Herbicides cause profound physiological changes in plants, which may alter their susceptibility to soilborne pathogens (Duke et al., 2007; Hale et al., 1981). Herbicide-stressed plants liberate more root exudates and the chemical nature of these leaked root components can stimulate or inhibit pathogen propagule germination (Brown and Curl, 1987; Lee and Lockwood, 1977). Alternatively, some herbicides can reduce disease severity, as reported for lactofen and *Sclerotinia sclerotiorum* in soybeans (Dann et al., 1999; Nelson et al., 2002). The mild oxidative stress resulting from PPO-inhibiting herbicide uptake can induce the synthesis of antimicrobial phytoalexins (Landini et al., 2003), but the magnitude of responses is likely dependent on a combination of factors including the pathosystem and crop stage under evaluation. There have been studies demonstrating PRE herbicides do not predispose seedlings to infection, particularly in cotton (*Gossypium hirsutum* L.) and soybean (Agamalian, 1964; Bauske and Kirby, 1992; Heydari and Misaghi, 1998), but controversial evidence has also been documented under field and controlled conditions (Bowman and Sinclair, 1989; Bradley et al., 2002; Carson et al., 1991; Espinoza et al., 1968; Harikrishnan and Yang, 2002; Neubauer and Avizohar-Hershenson, 1973). Specifically for residual PPO-inhibiting herbicides applied to soils, Daugrois et al. (2005) reported that sulfentrazone and flumioxazin resulted in increased colonization of sugarcane (*Saccharum officinarum* L.) roots by *Pythium tolurosum*. Conversely, Wilcut et al. (2001) reported a lack of interaction between flumioxazin and foliar and soilborne diseases in peanuts (*Arachis hypogaea* L). More recently, Priess et al. (2020) observed a greater incidence of *Pythium* stem rot resulting from flumioxazin application in one growing season. However, flumioxazin did not affect colonization rates of *Fusarium*, *Macrophomina*, and *Rhizoctonia*, nor affected plant density or yield across two environments (Priess et al., 2020).

Given the importance of PRE herbicides to weed management in soybeans and recurrent inquiries regarding synergism between PPO-inhibiting herbicides and early-season soilborne diseases (Giesler, 2017; Jhala, 2017; Wise et al., 2015), more research is needed to address the agronomical relevance of this potential interaction under field conditions. This study reports on the effect of a single mode of action, soil-applied residual PRE herbicides on the severity of soybean seedling diseases, injury, plant height, population, biomass, and yield across multiple environments prone to disease development in optimal and late-planted conditions.

2. Material and methods

2.1. Field experimental description

Six field trials were established in Nebraska near Mead and Lincoln in 2017, and near Mead, Bruno, Tekamah, and Arizona in 2018 (Table 1). All environments were previously cultivated with corn and were selected based on the history of soybean seedling diseases. Experimental plots were 5.18 m long by 3.04 m wide and consisted of four soybean rows spaced 0.76 m apart and at a density of 308,881 seeds ha^{-1}. No fungicide or insecticide treatments were applied to seeds and the planting date was typical of an optimum to a late plating for the region, between mid-May to early June across both years (Table 1). The experimental design was a randomized complete block design with four replications, however, the treatment design differed across growing seasons. In 2017, a two-way factorial design between PRE herbicides and soybean varieties Pioneer P28T08R (tolerant to PPO-inhibitors & metribuzin; *Rps*1k gene) and Pioneer P22T41R2 (sensitive to PPO-inhibitors & metribuzin; *Rps*1k gene) (DuPont Pioneer, Johnston, IA) was implemented. In 2018, only one variety, Asgrow AG27x8 (lacking herbicide tolerance ratings; *Rps*1c gene) was selected for all locations. PRE herbicide treatments consisted of (i) chlorimuron-ethyl (Classic 25DF, DuPont, Wilmington, DE) at 44 g ai ha^{-1}; (ii) metribuzin (Sencor 75 DF, Bayer CropScience, Research Triangle Park, NC) at 560 g ai ha^{-1}; (iii) saflufenacil (Sharpen, BASF, Research Triangle Park, NC) at 25 g ai ha^{-1}; (iv) sulfentrazone (Spartan 4F, FMC, Philadelphia, PA) at 350 g ai ha^{-1}; and (v) flumioxazin (Valor SX, Valent USA., Walnut Creek, CA) at 90 g ai ha^{-1}. A non-treated control was also included for comparison. PRE herbicide treatments were applied within 2 days after planting (DAP) and before soybean emergence using a CO_2–pressurized backpack sprayer equipped with a six-nozzle boom fitted with XR8002VS flat-fan nozzles (TeeJet Technologies, Spraying Systems Co., Wheaton, IL) spaced 50.8 cm apart. The spraying system was calibrated to deliver 140.3 L ha^{-1} at 275 kPa at a constant speed of 6.4 km h^{-1}. Experimental units were maintained weed-free throughout the season by hand-weeding, hoeing, and/or one or two applications of glyphosate (Roundup Power Max, Monsanto, St. Louis, MO) at 1140 g ae ha^{-1} + ammonium sulfate (N-Rich, American Plant Food Co., Galena Park, TX) at 2% by weight over the entire experimental area, shortly after planting but before emergence (VE) (Fehr et al., 1971) and after disease evaluation, between the fourth to sixth-trifoliate stages (V4–V6).

2.2. Data collection

Soybean injury was assessed visually between 10 and 15 DAP when seedlings were at the VE–VC growth stage. Injury was assessed on a 0–100% scale, combining the proportion of necrotic tissue on cotyledon and hypocotyl, seedling stunting, and yellowing of unifoliate leaves over the entire plot. Seedling root rot severity was assessed at 16 to 21 DAP when seedlings were at the V1–V2 growth stage. Six seedlings randomly selected from the outermost, non-harvested rows were dug, then soaked in water for approximately 20 min, and gently washed until coarse soil particles were removed. The proportion of root tissue with darkened, soft lesions was visually estimated for each plant using a 0–10 scale adapted from Bates et al. (2008) and a graded rating board. Plot-level disease severity indexes (DSI; 0–100%) were calculated using the formula: DSI $= \sum(\textit{severity rating x plants per rating})/(\textit{total plants} \text{ x } 10)$, adapted from Harveson et al. (2005). Shoot and root fresh biomass were recorded from the same six seedlings previously rated by cutting plants at the cotyledonary node and weighing plant parts separately. Plant

Table 1
Description of experimental sites and chronogram of field activities.

Year	Environment	GPS coordinates	Tillage	NWS[a]	Soil parameters						Planting date
					Type[b]	Sand	Silt	Clay	OM[c]	pH	
						(%)			dag kg^{-1}		
2017	Lincoln	40.861614, −96.595594	No-till	1-km	Kennebec silt loam 0–1% slope	17	51	32	3	6.9	31 May
	Mead	41.155339, −96.422101	Disked	9.4-km	Filbert silt loam 0–1% slope	15	47	38	2.4	5.9	1 Jun
2018	Tekamah	41.755558, −96.176062	No-till	1-km	Luton silty clay 0–1% slopes	17	16	67	5.4	6.2	18 May
	Arizona	41.792885, −96.139346	No-till	7.8-km	Haynie silt loam 0–2% slopes	19	36	46	3.4	7.6	18 May
	Mead	41.182523, −96.459948	No-till	5.7-km	Filbert silt loam, 0–1% slopes	17	48	35	4.7	6.8	6 Jun
	Bruno	41.293432, −96.916723	No-till	10-km	Zook silty clay loam 0–2% slopes	14	53	33	3.2	6.8	6 Jun

[a] NWS: nearest public weather station.
[b] Soil type obtained from Soil Survey of USDA Natural Resources Conservation Service (www.websoilsurvey.sc.egov.usda.gov).
[c] Organic matter content.

height was measured on six plants within each plot at the V1–V2 growth stage and stand counts were taken at two intervals, during the V1–V2 growth stages and before harvest, by counting the number of living plants in 3.05 m sections of the two innermost rows. Soybean grain yield was determined by harvesting 4.5 m of the two innermost rows within each plot using a small-plot combine (Almaco SPC20, Almaco, Nevada, IA) equipped with a grain gauge and handheld computer and grain yield was adjusted to 13% moisture.

2.3. Composition of root-associated organisms

The composition of filamentous fungal and oomycete organisms associated with symptomatic roots was evaluated from seedling roots previously rated at each environment. Roots were brought to the laboratory in coolers and surface disinfected in a 0.5% sodium hypochlorite solution for approximately 1.5 min, double-rinsed with tap water, and air-dried in a sterile laminar flow cabinet for 20–30 min. Isolations were performed with one-to-two lateral and taproot fragments ~2 cm long per plant, collected on the same day of sampling. Symptomatic root fragments were placed onto 100 mm diameter Petri dishes containing the following isolating media: (i) water agar at 20 g L^{-1}; (ii) water agar at 20 g L^{-1} + streptomycin (Sigma-Aldrich, St. Louis, MO) at 0.03 g L^{-1}; (iii) cornmeal agar (Difco, Sparks, MD) at 20 g L^{-1} + pentachloronitrobenzene (Sigma-Aldrich, St. Louis, MO) at 0.054 g L^{-1} + benomyl (Sigma-Aldrich, St. Louis, MO) at 0.01 g L^{-1} + spiramycin (Fisher Scientific, Pittsburgh, PA) at 0.005 g L^{-1}; and (iv) PBNIC V8 agar with rifampicin (Fisher Scientific, Pittsburgh, PA) added at 0.01 g L^{-1} (Dorrance et al., 2008). Twelve Petri dishes of each medium and four pieces of symptomatic root tissue were used per plate, resulting in 192 total possible isolates per environment. Culture plates were incubated for 3–12 days at 20 °C and checked daily for hyphal growth. Single pure isolates were obtained by sub-culturing hyphal growth onto a fresh Petri dish containing the same medium. Each sub-cultured isolate was examined microscopically and identified tentatively to the genus based on hyphal morphology, culture, and spore characteristics (Watanabe, 2010).

2.4. Statistical analyses

Statistical analyses were performed using R software (version 4.1.2) (R Core Team, 2021). Normality and variance homogeneity were tested via Shapiro-Wilk and Levene's tests with *'stats'* and *'car'* (version 3.0.12) packages, respectively, for each variable before ANOVA. Analyses were conducted separately for each environment due to differences in treatment design across years. Soybean density, biomass, height, DSI, and yield responses were fitted using a mixed-effect model with lmer function from *'lme4'* package (version 1.1.27.1). Soybean injury was fitted using a generalized linear mixed model with a beta distribution (family logit) from the package *'glmmTMB'* (version 1.1.2.3) (Stroup, 2015). Varieties and PRE herbicides were treated as fixed effects and blocks as random effects. All fitted models were evaluated via ANOVA with type III sum of squares using the *'car'* package and means separated using Tukey's honest significant difference (HSD) test from *'multcomp'* package (version 1.4.17) package when ANOVA indicated statistically significant effects ($P \leq 0.05$). At Tekamah in 2018, 5 out of 24 experimental units were removed from analysis due to varietal misplacement at planting. Abundance of filamentous organisms recovered from roots was evaluated through log-linear models using the *loglm* function of the package *'MASS'* (version 7.3.54) and the hypothesis of independence was accessed through the Likelihood ratio Chi-Square (LR χ^2) test. Initially, a log-linear model was fit to the global contingency table depicting six environments and four primary soybean root pathogenic genera *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia* plus the category others, which represented hereof all secondary pathogenic, non-pathogenic, and contaminants recovered. Orthogonally structured contrasts were created by partitioning the degrees of freedom from the global contingency table and subsequent LR χ^2 tests performed to provide further insights on the composition of isolates across groups and environments.

3. Results

3.1. Soybean injury, height, and density

The ANOVA results for PRE herbicides, varieties and their interaction are summarized for all response variables presented herein (Table 2). In general, PRE herbicide-associated injury levels were greater in 2018 than in 2017 although some variation also occurred within 2018 environments. No significant ($P \leq 0.05$) PRE herbicide or variety x PRE herbicide interaction effects were detected for soybean injury in 2017 (Table 2). A relatively small and yet significant difference in injury levels between PPO-sensitive P22T41R2 and the PPO-tolerant P28T08R cultivars was observed in Lincoln but not in Mead (data not presented). In contrast to 2017, more cotyledon and hypocotyl injury was observed across some 2018 environments. At Tekamah, saflufenacil resulted in >80% more soybean injury compared to metribuzin (Table 3), following precipitation and a brief decline in soil temperature after planting (Supplementary Fig. 1). Despite the prevalence of conducive environmental conditions, no differences in soybean injury were observed in Arizona (Table 3). At Mead in 2018, saflufenacil and

Table 2
Analysis of variance *P* values for soybean injury, disease severity index (DSI), soybean fresh shoot and root biomass, plant height, population density, and grain yield as affected by soil-applied PRE herbicides, varieties, and their interaction across environments.

Source of variation			Soybean injury	DSI	Biomass		Plant height	Plant population	Grain Yield
Year	Environment	Treatments			Shoot	Root			
					P-value[a]				
2017	Lincoln	Herbicide (H)	0.34	0.56	0.75	0.54	0.26	0.83	0.34
		Variety (V)	0.01	0.48	0.96	0.80	0.08	<0.01	0.49
		H x V	0.64	0.53	0.29	0.93	0.39	0.71	0.60
	Mead	H	0.47	0.92	0.91	0.18	0.37	0.76	0.61
		V	0.12	0.53	0.66	0.23	<0.01	<0.01	0.14
		H x V	0.98	0.21	0.91	0.45	0.03	0.50	0.89
2018	Tekamah	H	0.01	0.07	0.86	0.76	0.36	0.22	0.40
	Arizona	H	0.56	0.49	0.56	0.72	0.48	0.70	0.71
	Mead	H	0.06	0.32	–	0.01	0.58	0.99	–
	Bruno	H	0.11	0.30	0.07	0.15	0.34	0.21	0.08

[a] "–" denotes data were not recorded.

Table 3
Least-square means[a] and standard error of soil-applied residual PRE herbicides on soybean injury, height, and density.

PRE herbicide	Lincoln	Mead	Tekamah	Arizona	Mead	Bruno
	2017		2018			
			Crop injury (%)			
Non-treated control	6.1 ± 1	6.3 ± 1	30.4 ± 6 ab	22.0 ± 5	11.6 ± 2	30.6 ± 2
Chlorimuron-ethyl	11.2 ± 2	7.0 ± 1	27.9 ± 5 ab	25.6 ± 5	6.0 ± 1	42.3 ± 1
Metribuzin	11.3 ± 2	5.0 ± 1	20.4 ± 4 a	19.7 ± 4	12.2 ± 2	38.8 ± 2
Saflufenacil	10.3 ± 2	7.2 ± 1	49.7 ± 8 b	22.4 ± 5	17.1 ± 3	33.8 ± 3
Sulfentrazone	9.6 ± 2	7.2 ± 1	24.2 ± 5 ab	11.7 ± 4	14.2 ± 2	35.8 ± 2
Flumioxazin	7.5 ± 1	9.5 ± 1	37.3 ± 5 ab	19.9 ± 4	11.2 ± 2	25.5 ± 2
			Plant height (cm)			
Non-treated control	7.1 ± 0.2	6.8 ± 0.1	5.6 ± 0.3	5.4 ± 0.2	10.2 ± 0.6	7.9 ± 0.3
Chlorimuron-ethyl	6.7 ± 0.2	6.8 ± 0.1	5.3 ± 0.3	5.7 ± 0.2	10.6 ± 0.6	8.8 ± 0.3
Metribuzin	7.3 ± 0.2	6.8 ± 0.1	5.4 ± 0.2	5.7 ± 0.2	10.4 ± 0.6	7.6 ± 0.3
Saflufenacil	7.3 ± 0.2	6.7 ± 0.1	4.7 ± 0.3	5.7 ± 0.2	11.6 ± 0.6	8.0 ± 0.3
Sulfentrazone	7.2 ± 0.2	6.5 ± 0.1	5.5 ± 0.3	5.0 ± 0.2	11.0 ± 0.6	8.2 ± 0.3
Flumioxazin	7.4 ± 0.2	6.5 ± 0.1	5.2 ± 0.2	5.7 ± 0.2	10.5 ± 0.6	8.6 ± 0.3
			Plant density (1000 x ha^{-1})			
Non-treated control	236.5 ± 11	250.2 ± 8	127.0 ± 21	221.1 ± 22	186.2 ± 12	88.8 ± 12
Chlorimuron-ethyl	245.9 ± 11	261.8 ± 8	100.0 ± 21	196.4 ± 22	184.6 ± 12	90.9 ± 12
Metribuzin	227.1 ± 11	262.1 ± 8	148.5 ± 17	200.2 ± 22	189.9 ± 12	83.9 ± 12
Saflufenacil	230.0 ± 11	256.7 ± 8	80.7 ± 27	202.3 ± 22	185.1 ± 12	100.6 ± 12
Sulfentrazone	225.7 ± 11	262.1 ± 8	148.5 ± 21	222.4 ± 26	183.5 ± 12	75.8 ± 12
Flumioxazin	234.9 ± 11	250.2 ± 8	104.4 ± 17	242.7 ± 23	177.6 ± 12	116.2 ± 12

[a] Means in the same columns followed by the same letter are not statistically significant at 5% according to Tukey's HSD.

chlorimuron-ethyl resulted in 17.1 and 6% injury levels, respectively. No PRE herbicide effects were found in Bruno in 2018, despite moderately greater (>30%) injury levels (Table 3).

PRE herbicide had no effect on plant height, except in one environment (Table 2). At Mead in 2017, varietal height differences were found and the combination of sulfentrazone and PPO-sensitive variety resulted in the lowest plant heights among all treatments (data not presented). Additionally, soybean density taken at V1–V2 growth stage varied from 139,900 to 325,000 plants ha^{-1} and 40,900 to 307,800 plants ha^{-1} in 2017 and 2018, respectively, however, no effects of PRE herbicides were found (Table 2). Consistently higher population densities were observed for PPO-sensitive compared to the PPO-tolerant variety, with differences estimated at 58,400 and 31,600 plants ha^{-1} in Lincoln and Mead, respectively. Similar to 2017, no differences were observed in plant densities between PRE herbicides. Across environments, V1–V2 soybean density averaged 214,100, 184,500, 119,100, and 92,700 plants ha^{-1} in Arizona, Mead, Tekamah, and Bruno, respectively. Aside from these factors, variability in soybean densities seemed to be associated with exceptionally high pressure of seedling diseases in Tekamah in 2018 and soil crusting which limited uniform seedling emergence in Bruno in 2018. Soybean population density at harvest ranged between 146,300 and 290,600 and from 49,500 to 230,300 plants ha^{-1} in 2017 and 2018, respectively. No harvest plant density results are presented due to the lack of treatment differences.

3.2. DSI, plant biomass, and yield

Seedling root rot epidemics developed naturally across environments. Despite ranging from 21.7 to 46.7%, DSI was not affected by PRE herbicide, soybean variety, or their interaction in 2017 (Table 2). Numerically, DSI values were comparable between Lincoln and Mead, at 34 and 35.7%, respectively. Conversely, increased seedling root rot was observed in 2018 as a result of biological and environmental interactions with DSI ranging from 18.3 to 61.7% across experimental units. In Tekamah, the highest DSI environment, a marginal PRE herbicide effect was found ($P = 0.07$), with saflufenacil and metribuzin resulting in 53.3% and 31.2% DSI, respectively. Alternatively, no other differences were found between PRE herbicides and the non-treated control in this environment or elsewhere (Table 4). In other 2018 environments, DSI varied between 26.6–45% in Arizona, 18.3–43.3% in Mead, and 26.6–48.3% in Bruno, representing site-specific differences in seeding root rot. Contextually, these results do not support the hypothesis that single-active soil-applied PRE herbicides consistently interact with soybean seedling disease epidemics under conditions evaluated.

Regarding plant biomass, negligible PRE herbicide effects were found in the study, except in two environments (Table 2). Sulfentrazone,

Table 4
Least-square means and standard error of soil-applied residual PRE herbicides on soybean seedling root rot (DSI).

PRE herbicide	Lincoln	Mead	Tekamah	Arizona	Mead	Bruno
	2017		2018			
	DSI (0–100%)					
Non-treated control	31.9 ± 2	35.0 ± 2	41.7 ± 4	31.7 ± 2	27.9 ± 2	30.4 ± 2
Chlorimuron-ethyl	34.8 ± 2	36.7 ± 2	38.3 ± 4	31.7 ± 2	30.0 ± 2	35.8 ± 2
Metribuzin	34.8 ± 2	36.7 ± 2	31.2 ± 3	37.1 ± 2	27.9 ± 2	33.8 ± 2
Saflufenacil	31.7 ± 2	34.8 ± 2	53.3 ± 5	32.9 ± 2	22.9 ± 2	31.7 ± 2
Sulfentrazone	34.3 ± 2	34.6 ± 2	40.6 ± 4	31.1 ± 2	25.8 ± 2	38.3 ± 2
Flumioxazin	36.7 ± 2	36.2 ± 2	42.9 ± 3	33.8 ± 2	32.1 ± 2	37.9 ± 2

flumioxazin, and chlorimuron-ethyl resulted in ~20–30% less fresh root weight than metribuzin at Mead in 2018. A marginally significant ($P = 0.07$) effect was found at Bruno in 2018, with the non-treated control and flumioxazin resulting in the highest and lowest fresh root biomass, respectively. Additionally, grain yield ranged from 3409 to 5686 kg ha^{-1} in 2017, however, there were no consistent effects of PRE herbicide, soybean variety, or their interaction on grain yield (Table 2). Yield was 678.5 kg ha^{-1} greater in Mead than in Lincoln in 2017. Similar to other parameters evaluated, substantial yield differences, between 1373 and 5058 kg ha^{-1}, were observed across environments in 2018 (Fig. 1). A marginal effect ($P = 0.08$) was detected between PRE herbicides in Bruno with chlorimuron-ethyl yielding ~500 kg ha^{-1} less than the non-treated control, saflufenacil, and flumioxazin. In absolute terms, the average yield was 1656 and 1465 kg ha^{-1} greater in Mead than Tekamah and Bruno, respectively. Both sites had higher DSI values in comparison to other environments.

3.3. Composition of root-associated organisms

In conjunction, 417 isolates were recovered from symptomatic seedling root tissues. In 2017, 61 isolates representing 38% of the total were identified as primary soybean root pathogenic genera (*Fusarium*, *Phytophthora*, *Pythium*, and *Rhizoctonia*), whereas in 2018, 170 isolates (~66%) composed that group (Table 5).

LR χ^2 analysis between functional groups recovered demonstrated a highly diverse community structure across environments (LR $\chi^2_{20} = 124.0$, $P < 0.001$), indicating a variety of conditions evaluated for the effect of PRE herbicides on soybean seedling diseases. Exclusively among primary pathogenic genera, *Fusarium* was the dominant group representing 91.2% and 70.4% of relative frequency at Lincoln and Mead in 2017, respectively (Fig. 2A). *Fusarium* species structured 54.4% of isolates obtained from Tekamah in 2018, whereas the remaining composition was represented by *Phytophthora* (27.8%) and *Pythium* species (17.7%).

To further examine the distribution of isolate collection and their role, all six environments were grouped into low (<30%), intermediate (≥30 to < 40%), and high (≥40%) DSI classes. LR χ^2 test indicated that the variation in the number of isolates within primary pathogenic groups and DSI classes did not occur randomly, but was rather highly associated, using the field-specific data (Fig. 2B). Within the high DSI habitat, more oomycete isolates were recovered and represented 45.5% of the total major pathogenic group, as opposite to 54.5%, represented by *Fusarium* isolates. Conversely, at intermediate DSI environments, oomycetes structured 25.2%, while the majority of remaining isolates, more precisely 72.3% of the total in primary pathogenic genera, corresponded to *Fusarium* species. In relative terms, the lowest number of *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia* species were recovered from roots collected in the lowest DSI habitat. Analysis between oomycetes across DSI classes demonstrated (LR $\chi^2_2 = 0.29$, $P = 0.863$) that frequencies of *Phytophthora* and *Pythium* were, at least in its core, not associated with discrepancies in seedling root rot severity (Fig. 2C).

Overall, the relative frequency of *Pythium* spp. seemed to be low (<4%) across environments, except in Mead 2017 and Tekamah 2018, where it structured 7.3% and 13.7% of the recovered collection, respectively. Accounting exclusively for primary pathogenic genera, *Pythium* spp. represented 8.8%, 22.2%, 17.7%, 5.2%, 6.8%, and 4.6% of

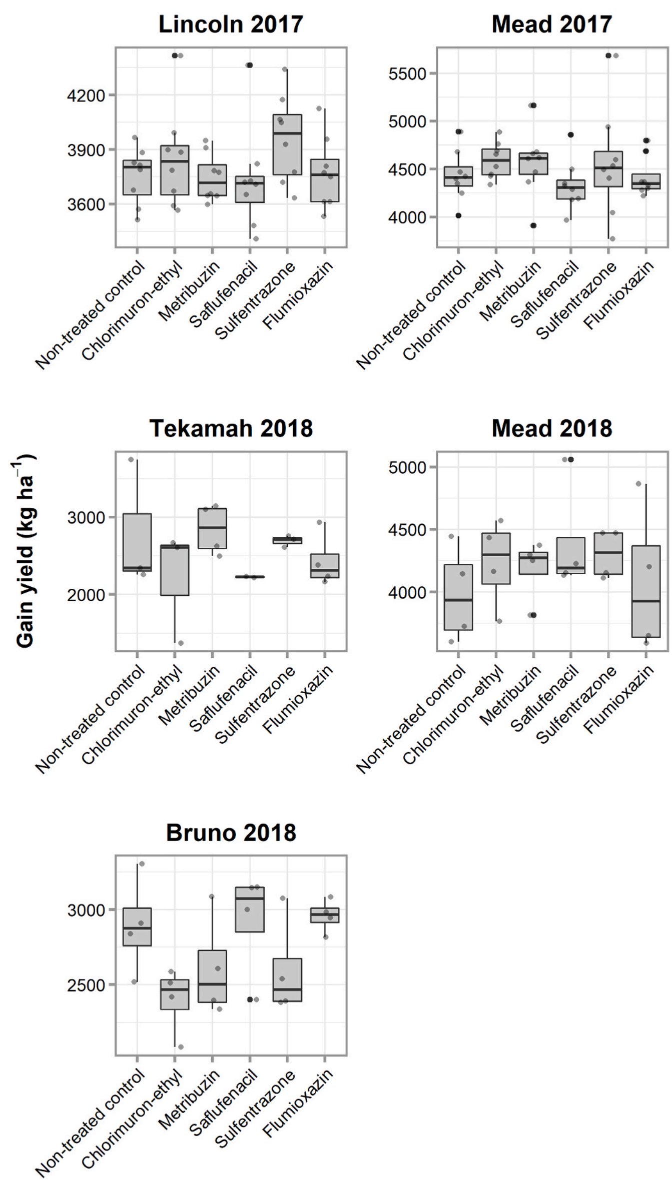

Fig. 1. Box plots summarizing the distribution of grain yield for different soil-applied residual PRE herbicides and a non-treated control. Dots represent each observation across all levels evaluated in each environment. Upper and lower edges of each box show the 75th and 25th percentiles of the data, respectively. Solid, horizontal lines within each box represent the median for each treatment.

Table 5
Data summary of disease severity index (DSI) and the number of isolates obtained from symptomatic soybeans.

	Lincoln	Mead	Tekamah	Arizona	Mead	Bruno
Disease (%), collection (n)	2017		2018			
DSI[a]	34 ± 6.5	35.6 ± 5.5	40.2 ± 8.6	33.1 ± 4.4	27.7 ± 5.9	34.6 ± 5.8
Isolates/collection[b]						
Fusarium spp.	31	19	43	17	19	22
Phytophthora spp.	0	0	22	0	5	19
Pythium spp.	3	6	14	1	2	2
Rhizoctonia spp.	0	2	0	1	3	0
others	44	55	23	21	26	17

[a] Arithmetic means and its ± standard deviation.
[b] Sum of the within-field collection of isolates obtained from symptomatic seedling root system plated onto four different media.

isolates collected at Lincoln and Mead in 2017, and Tekamah, Arizona, Mead, and Bruno in 2018, respectively. In contrast, *Phytophthora* was more geographically confined with presence detected in only half of the environments. However, when present, *Phytophthora* species constituted a relatively larger portion of community structure with 17.2%, 27.8%, and 44.1% of isolates in the primary pathogenic class at Mead, Tekamah, and Bruno in 2018, respectively. *Rhizoctonia* was the least predominant pathogenic genus recovered from symptomatic roots in this multi-environment study, comprising 1.4% of the total collection. Additionally, there was strong evidence that the abundance of primary pathogenic genera and others varied across environments (Fig. 2D), but no comprehensive identification of members in the group others was performed.

4. Discussion

The present multi-environment field study reports on potential interactions between soil-applied PRE herbicides and early-season soybean diseases under field conditions. Contextually, results do not indicate that chlorimuron-ethyl, flumioxazin, metribuzin, saflufenacil, and sulfentrazone applied PRE at labeled rates consistently affect soybean seedling root rot in optimal and late-planted scenarios. Conversely, community composition was functionally associated with the development of seedling disease epidemics. Noticeable variation existed in disease severity between environments and appeared to be related to field-specific elements, including the biological profile of organisms recovered from roots, rather than with PRE herbicide use.

The lack of consistent PRE herbicide-pathogen interaction is favorable to farmers, as soil-applied residual PRE herbicides are an important tool for the management of glyphosate-resistant weeds such as horseweed (*Erigeron canadensis* L.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), and waterhemp in soybean cropping systems. These findings, albeit speculatively, suggest PRE herbicide mixes may also not be consistently associated with soybean seedling disease at agronomically relevant scales, although more research is needed to make such conclusions. PRE herbicide mixes, particularly those containing acetolactate synthase (ALS) and PPO-inhibiting chemistries are a common component of weed management programs across many areas where soybeans are cultivated in the United States (Givens et al., 2009; Sarangi and Jhala, 2018).

Severe cotyledon and hypocotyl injury, typically associated with PPO-inhibiting herbicides were not widespread in the study, with exception of one environment where a combination of edaphic and environmental conditions was conducive for symptom expression. PRE herbicide injury to soybean can vary greatly depending on variety sensitivity, herbicide adsorptive behavior, and site characteristics such as soil moisture, pH, texture, and organic matter content (Gannon et al., 2014; Stewart et al., 2012). Low organic matter content and high

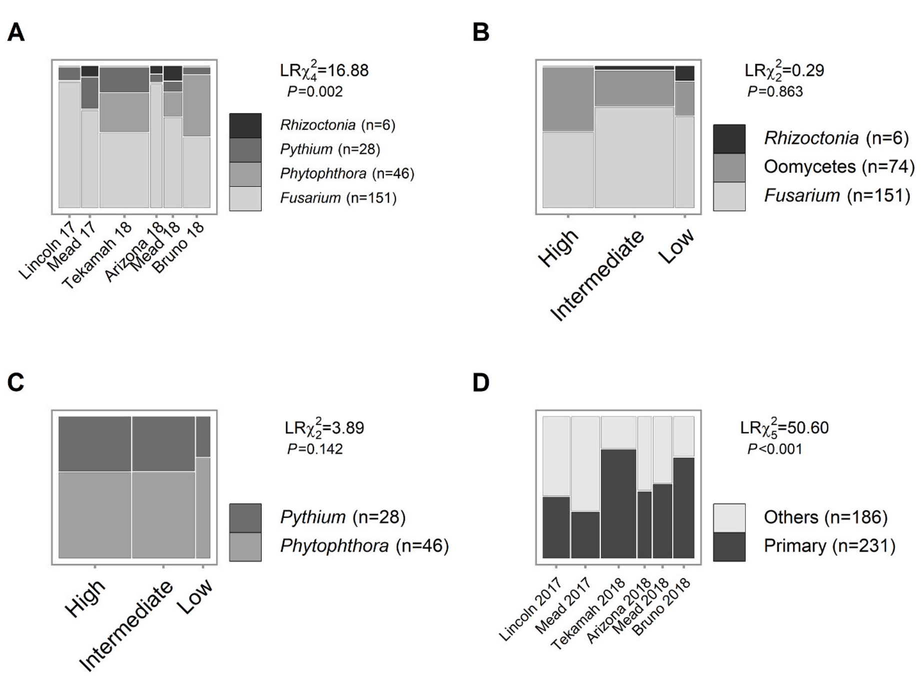

Fig. 2. Mosaic plots of the relative frequencies of isolates obtained from symptomatic soybean roots in Nebraska in 2017 and 2018. Likelihood Ratio (LR) χ^2 coefficients and *P*-values obtained from the log-linear model. Categorical factors varied between (**A**) primary pathogenic genera (*Fusarium*, *Phytophthora*, *Pythium*, and *Rhizoctonia*) and environments; (**B**) primary pathogenic group and disease severity index (DSI) classes; (**C**) primary pathogenic oomycetes and DSI classes; and (**D**) all groups including others (secondary pathogens, antagonistic, and contaminants) by environments. In total, 417 isolates were obtained from the six environments combined.

moisture increase the risk of injury by sulfentrazone (Wehtje et al., 1997). Poston et al. (2008) and Niekamp and Johnson (2001) documented greater injury levels resulting from PPO-inhibiting herbicide application under cool and wet soil conditions. Seedlings growing under favorable conditions can adequately metabolize herbicide active ingredients; however, their ability to metabolize these herbicides decreases under stressful conditions (Jhala, 2017). In this study, deleterious effects associated to PPO-inhibiting herbicides may have been minimized through the use of label recommended rates and the prevalence of warmer soil temperatures which offered adequate conditions for seedling emergence.

An area of study that is not entirely researched is the potential effects of PRE herbicides on soilborne pathogen infection and colonization. Priess et al. (2020) reported an increase in the incidence of *Pythium* spp. affecting soybeans following a flumioxazin application in one growing season but not in the following. Similarly, Bradley et al. (2002) observed enhanced root and hypocotyl rot caused by *Rhizoctonia solani* as a result of PRE herbicide application, but the magnitude and consistency of responses varied between years in field studies, suggesting the existence of complex interactions governing potential herbicide and disease susceptibility. Presumably, the predominance of *Fusarium*, *Phytophthora*, and to some extent *Pythium* species in this study, as opposed to *Rhizoctonia*, limited our comprehension of potential associations between PRE herbicides evaluated and that pathogen. However, in contrast to Priess et al. (2020), flumioxazin did not increase root rot in soybean seedlings in sites where *Pythium* and *Phytophthora* species were predominant. There is also some evidence that the effect of soil-applied PRE herbicides on seedling diseases is inoculum-dependent (Altman and Campbell, 1977; Carson et al., 1991), but our trials relied solely on natural soil infestation, and no attempt to determine pathogen inoculum in soil was performed. Notably, the application of saflufenacil, a PPO-inhibiting herbicide, seemed to enhance root rot compared to metribuzin in the highest disease severity environment with greater activity of oomycetes (*Phytophthora* and *Pythium* spp.), but this tendency was not mirrored in other agronomic parameters nor was replicated across environments evaluated. Additionally, no comparable differences in seedling root rot were found between PRE herbicides and the non-treated control.

Our study also reports on the role of community composition on seedling disease development. *Fusarium* spp. were dominant among common pathogenic genera with frequency varying between 23.1–42.5% of total isolates across environments, whereas *Pythium* spp., although recovered from all surveyed environments, represented only 2.5–13.7%. Given the ubiquitous presence of *Fusarium* spp. in soil and the ability to survive non-pathogenically as rhizosphere inhabitants and secondary invaders, some overrepresentation of their relative isolation frequency could be anticipated (Summerell et al., 2003). However, it is unknown why *Pythium* incidence was relatively low, despite field history and widespread occurrence. We assume that the higher temperatures during soybean emergence might have influenced the fitness of some *Pythium* spp., and in contrast, favored *Phytophthora* spp. (Rojas-Flechas et al., 2017a; Thomson et al., 1971). Despite aggregated occurrence, being isolated in half of the environments, *Phytophthora* spp. structured a large percentage (>20%) of total primary group isolates recovered, except one location with the lowest diseased root rot severity score. In addition to the primary pathogenic cohort, a range of other organisms, including *Trichoderma*, *Alternaria*, *Aspergillus*, and *Mortierella* species were recovered from soybean symptomatic roots in this study. Yet, efforts were directed toward the identification of the major pathogenic group which historically has been the predominant early-season soybean pathogens in Nebraska (Giesler et al., 2012; Parikh et al., 2018). In addition, it is important to address that no attempts to determine the pathogenicity of isolates obtained were performed, although literature suggests that not all isolates belonging to a pathogenic genus can necessarily cause disease (Coffua et al., 2016).

From a weed management perspective, using multiple residual PRE herbicides as part of a weed management and resistance mitigation program is beneficial, but further research is needed to generate adequate agronomic recommendations about PRE herbicide use in saturated and cool soil conditions, which are conducive to PPO-inhibiting herbicide injury and soybean seedling infection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2022.105987.

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