



# Influence of PRE-Emergence Herbicides on Soybean Development, Root Nodulation and Symbiotic Nitrogen Fixation<sup>1</sup>

## Take Home Message

- PRE-emergence (PRE) herbicides are important tools for control of troublesome weed species with extended emergence window such as waterhemp.
- Early-season herbicide injury and potential for negative impacts on Rhizobia root nodulation and subsequent symbiotic nitrogen fixation are concerns for soybean producers adopting PRE herbicides.
- Only sulfentrazone reduced soybean green canopy vegetation at the VC growth stage; however, none of the PRE herbicides evaluated in this greenhouse study had an impact on soybean canopy at the V2 growth stage, and soybean development, root nodulation, or nitrogen fixation at the R2 growth stage.

## Introduction

PRE-emergence (PRE) herbicides are recommended in soybean production systems for management of weed species with extended emergence window. Additionally, the use of PRE herbicides is considered a crucial component for management of glyphosate-resistant (GR) weeds. Due to the widespread prevalence of GR weeds and limited effective POST herbicide options in soybean, the use of PRE herbicides has become a standard recommendation for weed management in the US (Norsworthy et al., 2012). Early-season soybean injury due to PRE herbicide applications is a common concern amongst growers (Mahoney et al., 2014a, 2014b). The inoculation of soybean seeds with the Rhizobia bacteria *Bradyrhizobium japonicum* (Kirchner) Jordan, is a common practice in soybean production as these bacteria symbiotically colonize soybean roots and fix atmospheric nitrogen (N) for soybean plants (Mohammadi et al., 2012; Zimmer et al., 2016). There has been limited research investigating the impact of PRE herbicides on this symbiotic relationship (Chikoye et al., 2014; Aliverdi and Ahmadvand, 2018). If PRE herbicides negatively impact soybean development and root nodulation, symbiotic N fixation may be decreased and could negatively affect soybean grain yield and soil N availability for subsequent crops.

## Experiment Overview

In 2019 the UW-Madison Cropping Systems Weed Science Lab conducted a greenhouse experiment evaluating the influence of 11 commonly used PRE-emergence herbicides on soybean canopy, soybean root and shoot development, Rhizobia root nodulation, and symbiotic nitrogen fixation (description below).

## Objective

- Investigate the influence of 11 commonly used PRE herbicides on soybean growth and development, Rhizobia root nodulation, and symbiotic nitrogen fixation

Table 1: Herbicide treatment information for the greenhouse experiment conducted at the University of Wisconsin-Madison Walnut Street Greenhouse in Madison, WI in 2019.

Herbicide	Trade Name	Company	Group (SOA) <sup>a</sup>	Field Rate
chlorimuron-ethyl	Classic <sup>®</sup>	Corteva	ALS (2)	3.0 oz ac <sup>-1</sup>
cloransulam-methyl	FirstRate <sup>®</sup>	Corteva	ALS (2)	0.6 oz ac <sup>-1</sup>
imazethapyr	Pursuit <sup>®</sup>	BASF	ALS (2)	4.0 fl oz ac <sup>-1</sup>
metribuzin	Tricor <sup>®</sup> DF	UPL	PSII (5)	0.67 lb ac <sup>-1</sup>
flumioxazin	Valor <sup>®</sup> SX	Valent	PPO (14)	3.0 oz ac <sup>-1</sup>
saflufenacil	Sharpen <sup>®</sup>	BASF	PPO (14)	1.0 fl oz ac <sup>-1</sup>
sulfentrazone	Spartan <sup>®</sup>	FMC	PPO (14)	8.0 fl oz ac <sup>-1</sup>
acetochlor	Warrant <sup>®</sup>	Bayer	VLCFA (15)	1.5 qt ac <sup>-1</sup>
dimethenamid-P	Outlook <sup>®</sup>	BASF	VLCFA (15)	18 fl oz ac <sup>-1</sup>
pyroxasulfone	Zidua <sup>®</sup>	BASF	VLCFA (15)	3.0 oz ac <sup>-1</sup>
S-metolachlor	Dual II Magnum <sup>®</sup>	Syngenta	VLCFA (15)	1.67 pt ac <sup>-1</sup>

<sup>a</sup> Site of action (SOA), Acetolactate synthase (ALS)-, photosystem II (PSII)-, protoporphyrinogen oxidase (PPO)-, and very-long-chain fatty acid (VLCFA)-inhibiting herbicides.

## Materials and Methods (Technical Description)

A greenhouse experiment was conducted in 2019 at the Walnut Street Greenhouse, University of Wisconsin-Madison, in Madison, WI. The soil used in this experiment [silt loam (16% sand, 61% silt and 23% clay), pH of 6.9 and 6.4% organic matter (OM)] was collected from a certified organic field (no history of synthetic herbicide use) at UW-Madison Arlington Agricultural Research Station. The experimental unit consisted of a 2.6 gal pot filled with the field soil. The soil was not fertilized during the greenhouse experiment. Soybean seeds of variety AG24X7 (Bayer Crop Science, St. Louis, MO), were inoculated with *B. japonicum* (Cell-Tech Liquid, Bayer Crop Science, St. Louis, MO) at the rate of 4.3 fl oz inoculant per 100 lb seeds. Six seeds were sown per experimental unit (2 in depth) following inoculation with *B. japonicum*. To standardize comparisons amongst treatments, experimental units were thinned to a final density of 4 plants per experimental unit, 7 days after planting (thinned plants were randomly selected). PRE herbicides were applied one day after planting the soybean seeds using a research track sprayer equipped with a TP8002E (Teejet, Springfield, IL) nozzle calibrated to deliver 15 gal ac<sup>-1</sup>.

**Herbicide treatments are provided in Table 1.** Experimental units were watered to field capacity immediately following herbicide application and repeated daily for the remainder of the experiment. The experiment was conducted in a randomized complete block design with six replications and replicated twice over time (14 days apart).

Canopy cover (%) was measured 10 and 20 days after treatment (DAT; when the crop reached the VC and V2 growth stages, respectively) by taking photos with the Canopeo phone application (www.canopeoapp.com; Canopeo Software, Oklahoma State University, Division of Agricultural Sciences and Natural resources and the Soil Physics, Oklahoma, OK, USA). Soybean root and shoot biomass (g plant<sup>-1</sup>), root nodulation (# of nodules plant<sup>-1</sup>, nodule diameter and nodule activity), and symbiotic N fixation [acetylene reduction assay (ARA), natural abundance relative to atmospheric N (<sup>15</sup>N), and percentage of plant N derived from the atmosphere (%Ndfa)] were assessed at the R2 growth stage.

**Statistical analysis – R 3.5.1** Linear mixed models were fit to soybean root and shoot biomass, root nodulation, and symbiotic N fixation datasets and were analyzed and subjected to ANOVA. Canopy cover (%) were subjected to ANOVA using the beta distribution (family logit) program. For analysis of all response variables, herbicide treatments were considered as fixed effects and replications nested within experimental runs were treated as random. Means were separated when herbicide treatment effect was less than P = 0.05 using Fisher's protected least-significant difference.

Table 2: Soybean canopy (% green canopy cover plant<sup>-1</sup>) assessed at the VC (10 DAT) and V2 (20 DAT) soybean growth stages, root and shoot biomass (g plant<sup>-1</sup>) and number of nodules per plant assessed at the R2 growth stage (45 DAT) from the greenhouse experiment conducted at the University of Wisconsin-Madison Walnut Street Greenhouse in Madison, WI in 2019.<sup>abc</sup>

Herbicide	VC (10 DAT)	V2 (20 DAT)	Root Biomass	Shoot Biomass	Nodules Plant <sup>-1</sup>
untreated control	3.34 (2.87-3.90) abc	6.45 (5.70-7.29)	0.83 (0.65-1.00)	5.0 (4.3-5.6)	51 (38-62)
chlorimuron-ethyl	2.87 (2.43-3.37) cd	5.20 (4.54-5.95)	0.89 (0.71-1.06)	4.2 (3.6-4.8)	35 (23-46)
cloransulam-methyl	3.19 (2.73-3.73) abc	6.75 (5.98-7.60)	0.96 (0.78-1.13)	4.8 (4.1-5.3)	48 (36-59)
imazethapyr	3.56 (3.07-4.13) ab	6.53 (5.77-7.37)	0.94 (0.77-1.11)	4.8 (4.1-5.4)	49 (37-60)
metribuzin	3.17 (2.71-3.70) bc	6.08 (5.36-6.89)	0.73 (0.55-0.90)	4.5 (3.9-5.1)	55 (43-66)
flumioxazin	2.76 (2.34-3.25) cd	5.65 (4.96-6.44)	0.98 (0.80-1.15)	5.2 (4.5-5.8)	50 (38-61)
saflufenacil	3.34 (2.86-3.89) abc	6.16 (5.43-6.97)	0.80 (0.62-0.97)	5.0 (4.3-5.6)	47 (35-58)
sulfentrazone	2.41 (2.02-2.87) d	6.23 (5.49-7.05)	0.90 (0.72-1.07)	4.7 (4.1-5.3)	47 (35-58)
acetochlor	3.86 (3.30-4.51) a	6.49 (5.73-7.33)	0.81 (0.63-0.98)	4.7 (4.0-5.3)	44 (32-55)
dimethenamid-P	2.86 (2.43-3.37) cd	5.59 (4.90-6.37)	0.78 (0.60-0.95)	4.6 (3.9-5.2)	44 (32-55)
pyroxasulfone	3.58 (3.09-4.16) ab	6.42 (5.68-7.26)	0.85 (0.67-1.02)	4.6 (3.9-5.2)	50 (38-61)
S-metolachlor	3.63 (3.13-4.21) ab	6.10 (5.38-6.92)	0.85 (0.67-1.02)	4.5 (3.8-5.1)	41 (28-52)
P value	<0.001	0.096	0.207	0.454	0.154

<sup>a</sup> VC, unifoliate leaves; V2, two trifoliate; DAT, days after treatment

<sup>b</sup> Root and shoot biomass were evaluated at the R2 growth stage and reported in g plant<sup>-1</sup>

<sup>c</sup> Numbers reported are means with (Lower Confidence Interval and Upper Confidence Interval) at 5%. Means followed by the same letter are not different at the 95% level according to Fisher's LSD test.



Figure 1. Soybean plants at the VC growth stage assessment 10 days after treatment application. From left to right: untreated control (original photo, processed photo) and sulfentrazone (original photo, processed photo).

## Results and Discussion

The PRE herbicides tested in this study had minimal to no influence on early season soybean canopy development. Soybean canopy was only affected during the VC growth stage assessment ( $P < 0.01$ ; Table 2, Figure 1), when the sulfentrazone treatment reduced soybean canopy by 27% compared to the untreated control. Herbicide treatments had no impact on soybean canopy development at the V2 growth stage assessment when compared to the untreated control treatment ( $P = 0.096$ ; Table 2). Moreover, there was no impact of any PRE herbicide tested in this study on soybean root ( $P = 0.207$ ) and shoot ( $P = 0.454$ ) biomass per plant at the R2 growth stage (Table 2). Early season sulfentrazone injury in soybeans has been documented in previous research conducted by Arsenijevic et al. (2020) and Taylor-Lovell et al. (2001); however, soybeans overcame injury and no yield loss was observed in these field studies. The PRE herbicides tested in this study also had no impact on root nodulation including number of nodules per plant ( $P = 0.154$ , Table 2), nodule diameter ( $P = 0.362$ , data not shown), nodule activity (all nodules evaluated were pink in color thus considered fixing nodules, data not shown), and nodule biomass per plant ( $P = 0.203$ , data not shown) at the R2 soybean growth stage. Corroborating the root nodulation findings, the PRE herbicides also did not influence soybean N fixation according to the ARA ( $P = 0.254$ ),  $^{15}\text{N}$  ( $P = 0.215$ ) and %Ndfa ( $P = 0.215$ ) assessments (data not shown). These results indicate that the PRE herbicides evaluated herein can be applied to a silt loam soil with minimal concern of impact on Rhizobia nodulation and nitrogen fixation.

## Recommendation for Soybean Growers

According to the results of this study, the weed control benefits provided by PRE herbicides likely outweigh concerns regarding early-season injury, assuming that such herbicides are applied following their label requirements and the crop is established according to local best management practices. Moreover, these results showcase that single active ingredient PRE herbicides can be adopted in soybean with limited concern of impact on Rhizobia nodulation and nitrogen fixation, when applied according to their label and following local best management practices. Additionally, growers can opt for varieties with higher tolerance to PRE herbicides when such information is provided by seed companies as a means to reduce the likelihood of early-season crop injury. Future research should be conducted on herbicide premixes (multiple active ingredients) and under variable environmental conditions to determine any potential interaction between early season soybean injury and Rhizobia nodulation and nitrogen fixation.

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### Additional Resources

- [Residual Control of Waterhemp with PRE-emergence Herbicides in Soybean.](#)
- [Herbicide Comparison for Residual Waterhemp Control in Corn.](#)
- [2019 Wisconsin Weed Science Research Report.](#)
- [2020 Wisconsin Weed Science Research Report.](#)

