

Halauxifen-methyl preplant intervals and environmental conditions in soybean

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Research Article

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Abstract

Synthetic-auxin herbicides are often applied for horseweed control before soybean planting. However, certain days of planting interval must be maintained before soybean planting, depending on the product and rate used, because of potential crop phytotoxicity. Halauxifen-methyl is a new synthetic-auxin herbicide for horseweed control in preplant applications in soybean. Field experiments were conducted in 2015 and 2016 in Indiana to evaluate soybean phytotoxicity in response to applications of halauxifen-methyl (5 g ae ha⁻¹) at five preplant intervals (0, 1, 2, 3, and 4 weeks before planting [WBP]). In 2015, soybean phytotoxicity was not observed for any of the preplant intervals at any of the sites. In 2016, 0% to 15% phytotoxicity was observed at 14 d after planting (DAP) when halauxifen-methyl was applied at planting, 1 WBP, and 2 WBP at different sites. Soybean phytotoxicity was expressed in the unifoliolate leaves only at 14 DAP. However, the first trifoliolate did not show any injury symptoms at 21 DAP from any preplant application timing. Preplant application intervals for halauxifen-methyl did not affect soybean stand counts or grain yield in any site-year. Therefore, field results indicated that halauxifen-methyl applied alone can cause slight soybean phytotoxicity in preplant applications. In growth-chamber bioassays, reductions in soybean biomass, plant length, and emergence were accentuated at 30 C, compared with 20 or 15 C, when halauxifen-methyl was applied at 20 or 40 g ae ha⁻¹. These results contradict the currently held paradigm in which lower temperatures generally increase crop phytotoxicity levels to herbicide soil residual.

Introduction

Glyphosate-resistant (group 9) and acetolactate synthase (ALS)-resistant (group 2) horseweed populations are widespread in no-till production systems throughout the United States (Davis et al. 2008; Heap 2017; Kruger et al. 2009). In no-till glyphosate-resistant soybean, best management practices include the use of effective preplant herbicides to control emerged horseweed plants before planting plus residual herbicides to provide residual control (Loux et al. 2006).

Synthetic-auxin herbicides (group 4) such as 2,4-D and dicamba are used for horseweed control before planting in no-till glyphosate-resistant soybean (Bruce and Kells 1990; Loux et al. 2006, 2017; Wilson and Worsham 1988). However, these herbicides have inconvenient preplant restrictions that vary according to rate and formulation used, and can delay soybean planting under unfavorable weather conditions (Anonymous 2010a, 2010b, 2013; Thompson et al. 2007). Labels for 2,4-D products generally indicate a preplant interval of 7 to 15 d for ester formulations and 15 to 30 d for amine formulations for rates of 0.56 to 1.12 kg ae ha⁻¹ (Anonymous 2010b, 2013). Dicamba labels mandate a preplant interval of 14 to 28 d after a minimum accumulation of 2.5 cm of rainfall or overhead irrigation, at rates of 0.28 to 0.56 kg ae ha⁻¹ for all soybean varieties (Anonymous 2010a), except for approved dicamba formulations applied to dicamba-resistant soybean (Anonymous 2017a).

Overlooking preplant restrictions of synthetic-auxin herbicides in soybean can result in delayed seedling emergence, crop phytotoxicity, and yield reduction (Krausz et al. 1993; Thompson et al. 2007). Soybean phytotoxicity up to 40% and delayed soybean emergence up to 58% was reported by Krausz et al. (1993) for 2,4-D applications at soybean planting at 1.12 kg ae ha⁻¹. In addition, Thompson et al. (2007) reported that dicamba and 2,4-D applications at rates of 0.28 and 0.56 kg ae ha⁻¹, respectively, at soybean planting caused soybean phytotoxicity up to 73% for dicamba and 18% for 2,4-D at 35 d after planting (DAP).

Soybean seedling phytotoxicity due to herbicide residue in the soil has been documented in extension articles and research papers. The general paradigm is that phytotoxicity to preplant herbicide applications will increase under cool and wet soil conditions during soybean emergence, because of slower plant metabolism. This occurs for ALS- and protoporphyrinogen oxidase-inhibiting herbicides, as well as seedling root-growth-inhibiting herbicides (Hartzler 2017; Jhala 2017; Legleiter et al. 2014; Swantek et al. 1998; Taylor-Lovell et al. 2001). However, literature on the effects of soil moisture and temperature on soybean phytotoxicity

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Table 1. Description of soil properties for all sites.

Site ^a	Soil series description	pH	OM % meq 100 g ⁻¹	CEC
PPAC	Bourbon/Hanna series: fine-loamy over sandy texture with a very deep profile, somewhat poorly drained, and moderately rapidly permeable (Furr 1982)	6.3	2.2	6.0
SEPAC	Cobbsfork series: silt loam texture, known for ponding, wetness, low pH, crusting, and restricted permeability (Marshall 2011)	5.6	1.8	6.7
TPAC	Drummer soil series: silt-loam soil texture, deep profile, poorly drained, and moderately permeable (Ziegler and Wolf 1998)	6.2	2.9	13.3

^a Abbreviations: CEC, cation exchange capacity; OM, organic matter; PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); SEPAC, Southeast Purdue Agricultural Center (Butler, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN).

to preplant applications of synthetic auxins is very limited, although some herbicide labels indicate that cold and wet weather may enhance the possibility of phytotoxicity (Anonymous 2010a) or reduce herbicide efficacy (Anonymous 2010b). Therefore, understanding the effect of temperature and soil moisture on soybean phytotoxicity to preplant applications of synthetic auxins requires additional research.

Halauxifen-methyl is a new synthetic-auxin active ingredient for control of horseweed and other broadleaf weeds in preplant applications for corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean (Anonymous 2017b). In 2017, halauxifen-methyl received federal registration from the U.S. Environmental Protection Agency (Switalski et al. 2017). Product label indicates a preplant interval of 14 d in soybean at 5 g ae ha⁻¹ without environmental restrictions (Anonymous 2017c). Our objectives for this research were to evaluate the potential of halauxifen-methyl (5 g ae ha⁻¹) to cause soybean phytotoxicity under field conditions when applied at five different preplant intervals and investigate the relative importance of herbicide rate, temperature, and soil moisture on soybean phytotoxicity in a controlled environment.

Materials and Methods

Field Experiments

Research experiments were conducted during the 2015 and 2016 growing seasons at three locations in Indiana: Pinney Purdue Agricultural Center (PPAC; Wanatah: 41.44°N, 86.92°W), Southeast Purdue Agricultural Center (SEPAC; Butler: 39.03°N, 85.53°W), and Throckmorton Purdue Agricultural Center (TPAC; Lafayette: 40.29°N, 86.90°W). Each location provided diverse soil properties and environmental conditions (Table 1).

A stale seedbed system was used at the SEPAC site, whereas at TPAC and PPAC, soil preparation consisted of tillage approximately 30 d before soybean planting, with no soil disturbance after initial tillage. Tillage buries plant residue and promotes a less aggregated soil, which may increase herbicide degradation rates (Cheng and Lehmann 1985) and increase crop phytotoxicity after heavy rain events (via splashing).

Halauxifen-methyl (Elevore[®]; Dow AgroSciences LLC, Indianapolis, IN) was applied at five different preplant intervals (4, 3, 2, 1, and 0 WBP) at a rate of 5 g ae ha⁻¹; the study included a nontreated control. Methylated seed oil (MSO Ultra[™]; Precision Laboratories LLC, Waukegan, IL) was added at a rate of 1% vol/vol

according to label recommendations (Anonymous 2017c). Halauxifen-methyl was applied using a handheld CO₂-pressurized sprayer equipped with eight TeeJet XR 11002 nozzles (TeeJet Technologies, Glendale Heights, IL) spaced 38 cm apart, travelling at speed of 4.8 km h⁻¹ and calibrated to deliver 140 L ha⁻¹ spray solution at 131 kPa operating pressure.

Experimental areas were maintained weed-free throughout the growing season with multiple herbicide applications. Glyphosate (1,120 g ae ha⁻¹) plus ammonium sulfate (N-Pak[®] AMS; Winfield Solutions LLC, St. Paul, MN) at 2.5% vol/vol was applied at 1 WBP of soybean and in two POST applications. Paraquat (1,120 g ai ha⁻¹) plus crop oil concentrate (Prime Oil[®]; Winfield Solutions LLC, St. Paul, MN) at 1% vol/vol was applied at planting. Hand weeding of escapes occurred as needed.

Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 3 m wide by 9 m long. Glyphosate-resistant soybean varieties were planted in 76-cm rows at seeding rates that ranged from 259,000 to 420,000 seeds ha⁻¹, according to local practices at each site (Table 2).

Evaluations consisted of visual ratings (percentage) of soybean phytotoxicity and two soybean stand counts per plot (plants m⁻¹ of row) at 14, 21, and 35 DAP. Plots were harvested at the end of the growing season to evaluate the effect of halauxifen-methyl on soybean grain yield. Differences in soybean phytotoxicity, stand counts, and grain yield were determined with one-way ANOVA using PROC GLIMMIX in SAS, version 9.4 (SAS Institute Inc., Cary, NC) with mean separation using Tukey's honestly significant difference ($P \leq 0.05$).

Growth-Chamber Experiments

Soil residual bioassays were conducted in 2018 in a growth chamber (Conviron[®] PGR15; Controlled Environments Ltd., Winnipeg, Manitoba, Canada) using pregerminated soybeans as bioindicator plants to investigate the influence of herbicide rate, temperature, and soil moisture on crop phytotoxicity. Soybean seeds (Asgrow[®] 2933; Monsanto Co., St. Louis, MO) were placed in a tray with moist cloth for 48 h at 25 C to allow imbibition and initiate germination before the experiment setup.

The experimental design consisted of a full factorial design with three factors (temperature, soil moisture, and herbicide rate), 12 repetitions per combination of factors, and two runs ($n = 24$). Three temperatures (15, 20, and 30 C) were tested separately with constant light intensity (900 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and air relative humidity (70% \pm 5%), and a photoperiod of 14 h. In addition, three levels of soil moisture were tested (40%, 58%, and 75% of water-holding capacity by weight) and the rates of halauxifen-methyl were 0, 10, 20, and 40 g ae ha⁻¹. Halauxifen-methyl rates, soil moisture levels, and temperature levels tested in this experiment were selected on the basis of observations from a preliminary run (data not shown) to provide a range of environmental conditions while also allowing plant survival.

The field soil used in this experiment consisted of a loamy sand texture (83% sand, 10% silt, and 7% clay) with 2.2% organic matter, pH of 7.0, and cation exchange capacity of 9.6 meq/100 g, according to the soil analysis. The soil was air dried to constant weight and sieved using a \pm 2-mm mesh sieve and then mixed with different herbicide solutions or water. Herbicide solutions were adjusted to provide the desired herbicide rate (in g ae ha⁻¹) once mixed with air-dried soil (calculations were based on an acre furrow slice weighing 2 million pounds). Next, 144 g of treated soil was used to fill each 164-cm³ cone (Ray Leach SC-10 Super Cell Cone-

Table 2. Field preparation, soybean variety, and planting parameters for all site years.

Site year ^a	Soybean variety	Field preparation ^b	Planting date	Soil moisture	Seeding rate	Soil temperature	Planting depth
					seeds ha ⁻¹	C	cm
PPAC 2015	Becks 278R4	Tillage	May 29	Dry	259,000	23	5
PPAC 2016	Pioneer P28T08R	Tillage	May 27	Dry	395,000	21	5
SEPAC 2015	Asgrow 2933	No-till	May 22	Moist	259,000	17	3
SEPAC 2016	Asgrow 2933	No-till	May 25	Moist	346,000	26	5
TPAC 2015	Asgrow 2933	Tillage	May 22	Dry	346,000	18	5
TPAC 2016	Asgrow 2933	Tillage	May 16	Dry	420,000	16	4

^a Abbreviations: PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); SEPAC, Southeast Purdue Agricultural Center (Butler, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN).

^b Tillage occurred before first halauxifen-methyl application.

Table 3. Influence of halauxifen-methyl^a application timing on soybean phytotoxicity for all sites at 14 d after planting in 2016.

Preplant interval	Soybean phytotoxicity by site ^{b,c}		
	PPAC	SEPAC	TPAC
weeks before planting	%		
Nontreated control	0 b	0 a	0 b
4	1 b	0 a	0 b
3	1 b	0 a	0 b
2	15 a	1 a	4 a
1	13 a	4 a	5 a
0	0 b ^d	6 a	5 a
P value	<0.0001	0.1989	<0.0001

^a Halauxifen-methyl was applied at 5 g ae ha⁻¹.

^b Abbreviations: PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); SEPAC, Southeast Purdue Agricultural Center (Butler, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN).

^c Means followed by same letter within a column are not statistically different according to Tukey's honestly significant difference ($P \leq 0.05$).

^d The 0 weeks before planting treatment was applied 3 days after planting at PPAC in 2016.

tainers; Stuewe & Sons, Tanget, OR). One pregerminated soybean seed was placed in each cone and covered with 30 g of sand to prevent soil-surface crusting. Soil moisture was adjusted for each cone on the basis of weight. The weight of the herbicide solution added was considered when adjusting soil moisture. Cone water-holding capacity was determined by saturating air-dried soil and measuring the change in weight 24 h later to allow water drainage by gravity.

Soil moisture levels were maintained by weighing the cones daily and adding water to achieve desired weight. Each run of the experiment was maintained in the growth chamber until the nontreated plants reached a fully expanded unifoliate, which corresponded to a total of 7, 9, and 11 d for 30, 20, and 15 C, respectively.

Evaluations consisted of total plant length (roots plus shoots), plant biomass, and soybean emergence at harvest. A factorial ANOVA using PROC GLIMMIX in SAS for a type I test of fixed effects ($P \leq 0.05$) was conducted on plant length and biomass data. Outliers were removed for soybean biomass data on the basis of Cook's distance test for influential data points. Soybean emergence data, which were categorical (i.e., 0 or 1), were analyzed using PROC FREQ in SAS for a χ^2 test of interactions among herbicide rate, temperature, and soil moisture.

Results and Discussion

Field Experiments

Soybean phytotoxicity did not occur in 2015 for any of the halauxifen-methyl treatments at any site. In 2016, phytotoxicity was observed at all sites at 14 DAP (Table 3). However, differences

in environmental conditions between 2015 and 2016 (Figures 1 and 2) did not explain the cause of increased soybean phytotoxicity in 2016. The PPAC site had the highest levels of soybean phytotoxicity in 2016; however, this site was not the coldest, nor did it receive the highest amounts of rainfall compared with the other site-years. Therefore, growth-chamber experiments were conducted in 2018 to further investigate the influence of environmental conditions on the level of soybean phytotoxicity in response to halauxifen-methyl. Epinasty occurred in the field in the unifoliate leaves, resembling symptoms caused by 2,4-D in preplant applications. Halauxifen-methyl applied at 3 and 4 WBP caused 1% soybean injury at PPAC in 2016. The preplant interval of 2 WBP resulted in phytotoxicity of 15%, 1%, and 4% at PPAC, SEPAC, and TPAC, respectively. Applications at 1 WBP resulted in 13%, 4%, and 5% phytotoxicity for PPAC, SEPAC, and TPAC, respectively. Finally, applications at planting resulted in 0%, 6%, and 5% phytotoxicity for PPAC, SEPAC, and TPAC, respectively. Because of unfavorable weather conditions, the 0 WBP treatment at PPAC was delayed and applied three DAP, before soybean emergence. However, no soybean phytotoxicity was observed for this treatment.

Halauxifen-methyl half-life under field conditions is approximately 15 d, and soil mobility of halauxifen-methyl is limited (soil adsorption coefficient, $K_{oc} = 1,418 \text{ mL g}^{-1}$) (Shaner 2014). Therefore, positioning and/or solubilization of halauxifen-methyl molecules in the soil at the time of soybean emergence may influence the occurrence of phytotoxicity.

Soybean plants recovered from halauxifen-methyl phytotoxicity rapidly, and at 21 DAP, previously injured plants displayed no observable injury in the first trifoliate. Therefore, soybean

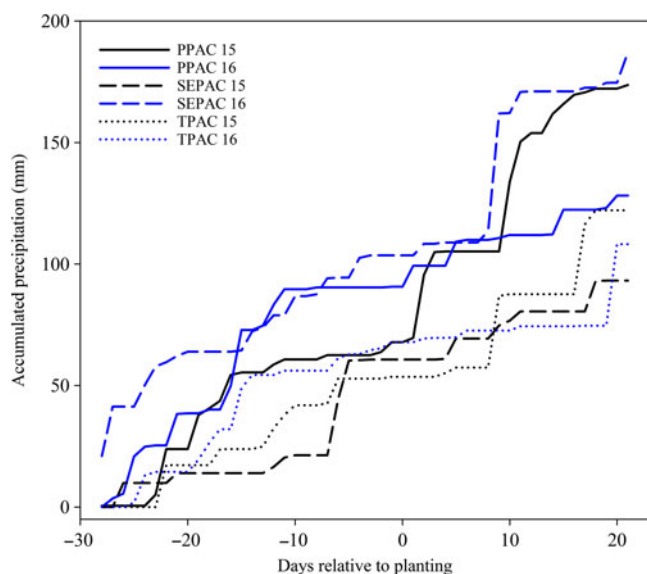


Figure 1. Daily accumulated precipitation (mm) from 28 d before planting to 21 d after planting for all sites in 2015 and 2016. Abbreviations: PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); SEPAC, Southeast Purdue Agricultural Center (Butler, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN).

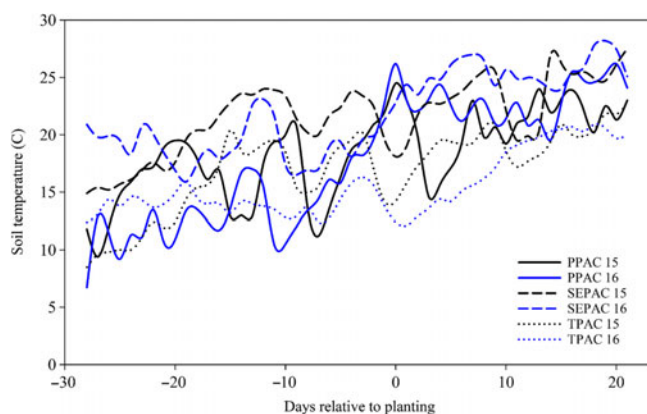


Figure 2. Daily soil temperature averages (C) from 28 d before planting to 21 d after planting for all sites in 2015 and 2016. Abbreviations: PPAC, Pinney Purdue Agricultural Center (Wanatah, IN); SEPAC, Southeast Purdue Agricultural Center (Butler, IN); TPAC, Throckmorton Purdue Agricultural Center (Lafayette, IN).

phytotoxicity symptoms were deemed negligible at 21 DAP (data not shown). Thompson et al. (2007) observed soybean phytotoxicity at 14 DAP up to 16%, 18%, and 31% for 2,4-D ester, 2,4-D amine, and dicamba applications at planting, respectively. In addition, soybean phytotoxicity increased or persisted for ratings at 35 DAP, up to 14%, 12%, and 73% for 2,4-D ester, 2,4-D amine, and dicamba, respectively. Studies performed by Krausz et al. (1993) showed soybean phytotoxicity up to 35% at 30 DAP for 2,4-D applications at 0.56 kg ae ha⁻¹ at planting and up to 18% at 45 DAP. Experiments including these three herbicides simultaneously under the same environmental conditions are necessary to make better comparisons.

Preplant application timings of halauxifen-methyl did not affect soybean stand counts or soybean grain yield for any of the sites and years evaluated (Table 4). Research has shown that 2,4-D applied at

Table 4. Influence of halauxifen-methyl^a application timing on soybean stand counts at 35 d after planting and soybean grain yield for all site years.

Preplant interval	Soybean stand ^{b,c}	Soybean grain yield
weeks before planting	plants m ⁻¹ of row	kg ha ⁻¹
Nontreated control	26a	4380a
4	27a	4400a
3	27a	4400a
2	26a	4510a
1	26a	4380a
0	26a	4460a
P-value	0.3237	0.3505

^a Halauxifen-methyl was applied at 5 g ae ha⁻¹.

^b Data pooled over six site years ($n=24$).

^c Means followed by same letter within a column are not statistically different according to Tukey's honestly significant difference ($P \leq 0.05$).

Table 5. Type I test of fixed effects for total soybean plant length in controlled environment experiment.

Effect ^a	Num DF ^b	Den DF	F value	P value
Herbicide rate	1	796	1332.27	<0.0001
Temperature	1	796	2.10	0.1478
Herbicide rate × temperature	1	796	29.18	<0.0001
Soil moisture	1	796	26.41	<0.0001
Herbicide rate × soil moisture	1	796	0.04	0.8457
Temperature × soil moisture	1	796	3.20	0.0741
Herbicide rate × temperature × soil moisture	1	796	3.67	0.0559

^a Data for two runs combined ($n=24$).

^b Abbreviations: DF, degrees of freedom; Den, denominator; Num, numerator.

Table 6. Type I test of fixed effects for total soybean biomass in controlled environment experiment.

Effect ^a	Num DF ^b	Den DF	F value	P value
Herbicide rate	1	785	198.72	<0.0001
Temperature	1	785	35.21	<0.0001
Herbicide rate × temperature	1	785	15.16	0.0001
Soil moisture	1	785	47.30	<0.0001
Herbicide rate × soil moisture	1	785	5.66	0.0176
Temperature × soil moisture	1	785	0.81	0.3686
Herbicide rate × temperature × soil moisture	1	785	0.03	0.8519

^a Data for two runs combined ($n=24$).

^b Abbreviations: DF, degrees of freedom; Den, denominator; Num, numerator.

planting can delay soybean emergence up to 50% at 0.56 kg ae ha⁻¹, and dicamba applied at 0.28 kg ae ha⁻¹ at planting can reduce yield up to approximately 55% (Krausz et al. 1993; Thompson et al. 2007).

Factors such as herbicide application rates, soil moisture and texture, temperature, precipitation, tillage, and planting depth can influence herbicide degradation rates and persistence in the soil (Cheng and Lehmann 1985). Experiments conducted over a wider range of soil and climatic conditions are necessary to determine the influence of environmental factors on the level of phytotoxicity that halauxifen-methyl may cause in the field. The growth chamber experiment discussed in the following section was conducted to additionally investigate the influence of these factors on herbicide phytotoxicity levels.

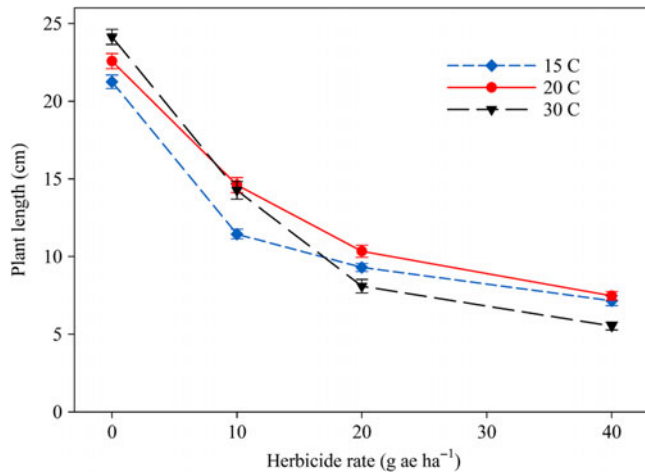


Figure 3. Influence of herbicide rate and temperature on soybean plant length in growth chamber experiment harvested at VC growth stage.

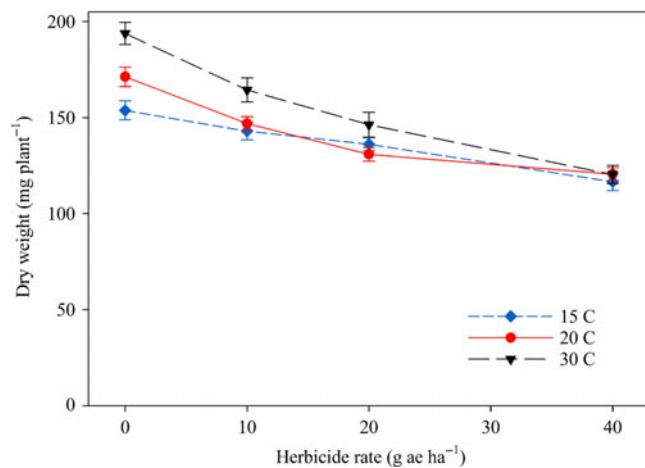


Figure 4. Influence of herbicide rate and temperature on soybean biomass in growth chamber experiment harvested at VC growth stage.

Growth-Chamber Experiments

Statistical analysis of the growth-chamber experiment showed an interaction between halauxifen-methyl rate and temperature ($P \leq 0.0001$) for soybean plant length (Table 5) and biomass (Table 6). Interaction plots (Figures 3 and 4) showed that greater plant length and biomass reduction occurred for soybean plants growing at 30 C than at 15 or 20 C as halauxifen-methyl rate increased. Furthermore, soybean emergence at 30 C was less than 55% for all moisture levels when halauxifen-methyl was applied at 20 and/or 40 g ae ha⁻¹, which did not occur at 15 or 20 C (Figure 5). The interaction between herbicide rate and temperature for soybean emergence data was also significant according to the χ^2 test ($P = 0.0003$; data not shown).

Although the statistical analysis also indicated an interaction between halauxifen-methyl rate and soil moisture for soybean biomass (Table 6), this interaction was not significant for plant length (Table 5) or soybean emergence (Figure 5). The main effects are not discussed, because of the occurrence of interactions.

Although the general paradigm is that soybean phytotoxicity to preplant herbicide applications will increase when plants emerge

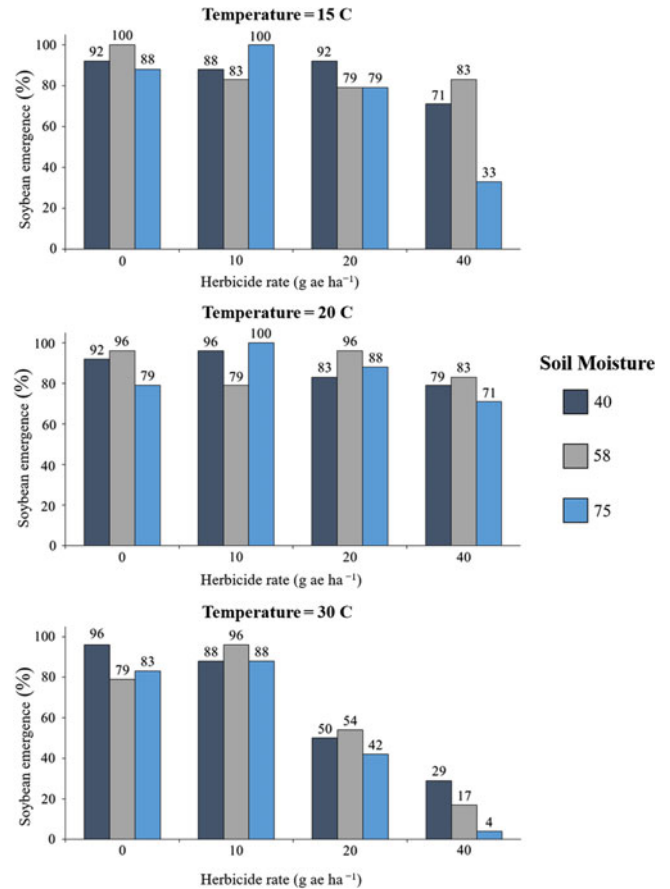


Figure 5. Influence of herbicide rate, temperature, and soil moisture on soybean emergence in growth chamber experiment harvested at VC growth stage.

under cool, wet conditions (Hartzler 2017; Jhala 2017; Legleiter et al. 2014; Swantek et al. 1998; Taylor-Lovell et al. 2001), data supporting this paradigm for synthetic-auxin herbicides are lacking. Literature on other species does exist, although it is not conclusive. Experiments conducted by Gauvrit and Gaillardon (1991) on corn (*Zea mays* L.) showed that cold stress reduced 2,4-D metabolism and increased phytotoxicity. In contrast, Shibasaki et al. (2009), in *Arabidopsis thaliana*(L.) Heynh., showed that cold temperatures inhibited root basipetal auxin transport, thus reducing auxin activity. Friesen and Dew (1966) investigated the influence of soil moisture and temperature on activity of 2,4-D, dicamba, and picloram in Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.] and showed that higher moisture increased phytotoxicity compared with low soil moisture, whereas temperature had little effect on phytotoxicity.

In conclusion, the results of this research contradict the currently held paradigm in which lower temperatures result in increased soybean phytotoxicity to preplant herbicide applications of synthetic auxins. High temperatures (30 C) resulted in greater reductions in plant length, biomass, and emergence in comparison with the nontreated control. Increased transpiration rates and herbicide uptake from the soil solution during emergence may explain reduced biomass and plant length as well as lower rates of soybean emergence at higher temperature levels. Additional research investigating the mechanisms of auxin uptake by soybean plants and the routes of metabolic degradation, as well as the environmental fate

of halauxifen-methyl after application, are necessary to further explain these results.

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