Does the Application of a Fungicide after a Herbicide Reduce Soybean Injury and Increase Yield?

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Abstract

A total of four field experiments were conducted during 2017, 2019 and 2020 in Ontario, Canada to determine if applying a fungicide 2 - 3 days after a herbicide, applied POST, reduces visible injury, increases crop vigour and increases yield of soybean. At 3 DAB (days after fungicide application), the POST application of glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr caused 0, 11%, 5%, 18%, 9% and 12% visible injury in soybean, respectively. The injury decreased over time with less than 5% injury at 8 WAB (weeks after fungicide application) in all treatments evaluated. The application of pyraclostrobin/fluxapyroxad after the application of herbicides evaluated did not reduce soybean injury. Soybean vigour with glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr applied POST without the fungicide application was 100%, 91%, 95%, 84%, 91% and 88%, respectively at 3 DAB. The soybean vigour increased over time to 95% - 100% at 8 WAB. The application of pyraclostrobin/fluxapyroxad after the herbicide application did not improve soybean vigour, except with thifensulfuron-methyl where soybean vigour was improved 6% when followed by pyraclostrobin/fluxapyroxad. There was no effect of herbicide and fungicide treatments on soybean yield except for thifensulfuron-methyl and imazethapyr without the fungicide treatments which reduced soybean relative yield 7% and 10%, respectively. The application of pyraclostrobin/fluxapyroxad after the application of imazethapyr increased soybean yield 3%. Based on these results, applying pyraclostrobin/fluxapyroxad fungicide 2 - 3 days after glyphosate, fomesafen, bentazon and cloransulam-methyl does not affect soybean injury, vigour or yield, but it can slightly enhance the vigour and yield of soybean when applied after thifensulfuron-methyl and imazethapyr.

Keywords

Glyphosate, Fomesafen, Bentazon, Thifensulfuron-Methyl, Cloransulam-Methyl, Imazethapyr, Pyraclostrobin/Fluxapyroxad, Vigour, Yield

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is an economically important crop produced in southwestern Ontario. In 2020, growers in Ontario seeded over 1.2 million hectares of soybean and produced nearly 4 million tonnes valued at over $1.6 billion making it the second most important grain cash crop grown after corn in the province in terms of total farm gate value [1]. Weeds and diseases if not managed can reduce the yield, quality, and profitability of soybean.

The common problematic weeds in Ontario include common lambsquarters (*Chenopodium album*), Canada fleabane (*Conyza canadensis*), common ragweed (*Ambrosia artemisiifolia*), eastern black nightshade (*Solanum ptycanthum*) and pigweeds (*Amaranthus spp.*) [2]. Soybean growers in Ontario often utilize glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr applied POST to manage these weeds [2]. However, some of these herbicides can cause transient crop injury which may affect soybean vigour and yield [2] [3].

Diseases of concern in soybean production under Ontario environmental conditions include septoria brown spot (*Septoria glycines*), phytophthora root and stem rot (*Phytophthora sojae*), powdery mildew (*Microsphaera diffusa*), downy mildew (*Peronospora manshurica*), brown stem rot (*Phialophora gregata*), sudden death syndrome (*Fusarium verguliforme*), white mould (*Sclerotinia sclerotiorum*) and Asian soybean rust (*Phakospora pachyrhizi*) [4] [5]. Soybean growers often use a relatively new class of strobilurin fungicides which are known as quinone-outside inhibitor (Qol) fungicides to control these diseases [6] [7]. Foliar applications of strobilurin fungicides after major abiotic stress events have been promoted to reduce soybean injury, increase vigour, improve plant health, and increase yield [7]-[14]. Strobilurin fungicides have been reported to cause physical changes which delay senescence [15] [16] [17] elevate phytohormones that decrease ethylene biosynthesis which can increase antioxidative enzymes and reduce free radical production [16] [18], and increase nitrate reductase activity [19] [20] [21] within higher plants. Other studies have suggested that strobilurin fungicides increase water use efficiency of plants under water deficit conditions [19] [21]. Effect of non-disease physiological effects in host plants have been reported to increase soybean yield [22] [23] [24].

Fungicide manufacturers and some researchers have documented crop yield increases following the foliar application of strobilurin fungicide (and some other fungicides) even when there is no disease is visibly present at the time of application [7] [24]. Some soybean producers use foliar fungicides in the absence of diseases to reduce crop stress and increase seed yield [24]. Fungicide application after a herbicide application may have the potential to reduce herbicide induced plant stress. This assumption has not been investigated for soybean production under Ontario environmental conditions. To our knowledge, there is no published information on the benefits of applying a foliar fungicide three days after the application of a herbicide on soybean injury, vigour and yield under Ontario environmental conditions. Lack of information, or incorrect information, leads to unnecessary pesticide applications which results in increased pesticide loading in the environment, increased pressure for the evolution of pest resistance, elevated crop production costs, and reduced net returns for soybean producers.

The objective of this research was to determine if applying a fungicide 2 - 3 days after a herbicide, applied POST, reduces visible soybean injury, increases crop vigour and elevates yield.

2. Materials and Methods

Four field experiments were conducted in Ontario, Canada, 1 in 2017 at the University of Guelph, Ridgetown Campus, Ridgetown, ON (42˚26'0"N, 81˚53'0"W); 2 in 2019 and 1 in 2020 at the University of Guelph, Huron Research Station near Exeter, ON (43˚19'1.2108"N, 81˚30'3.8736"E). The 3 experiments in 2019 and 2020 included two soybean cultivars (DR "DKC10-20" and GR "DKC27-12") in each plot. The experiment in 2017 included one soybean cultivar (DR "DKB10-01").

The experimental design was factorial, with herbicide treatment and fungicide treatment as the two factors. Field trials were established as RCBDs and each treatment (factor combination) was replicated four times. Treatments included a non-treated control, glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr applied POST alone and followed by pyraclostrobin/fluxapyroxad fungicide (2 to 3 days later) at rates listed in **Table 1**. Herbicide (including adjuvants used) and fungicides rates used were based on manufacturers' recommended rates in soybean in Ontario, Canada.

Plots were 3 m wide (4 rows spaced 75 cm apart) and 8 m long at Ridgetown and 10 m long at Exeter. Each plot consisted of two rows of glyphosate/dicamba-resistant (GDR) soybean (DeKalb "DKC10-20") and 2 rows of GR (DeKalb "DKC27-12") when there were 2 cultivars in each plot or 4 rows of DR ("DKB10-01") soybean when there was one soybean cultivar in each plot. Soybean was seeded to a depth of 4 cm in May to early June at a rate of approximately 380,000 seed ha^{-1}.

Herbicides were applied POST at V4-V5 soybean leaf stage followed by a fungicide POST application (2 - 3 days after herbicide application) with a CO_2-pressurized back-pack sprayer equipped with Hypro ULD120-02 nozzle tips (Hypro, New Brighton, MN) calibrated to deliver 200 $L \cdot ha^{-1}$ of water at 240 kPa. The spray boom was 2.5 m long with six ultra-low drift nozzles spaced 0.5 m apart

Table 1. Significance of main effects and interaction for soybean visible injury when treated with herbicides followed by a fungicide 2 - 3 days later in trials conducted at Ridgetown, ON (2017) and Exeter, ON (2019-2020), n = 4. Means for a main effect were separated only if the interaction involving the main effect was negligible[a].

Main effects		Visible Injury (%)				
		3 DAB	1 WAB	2 WAB	4 WAB	8 WAB
Herbicide treatment	*Rate* (g ai/ha)					
No herbicide		0 a	0 a	0 a	0 a	0 a
Glyphosate	900	0 a	0 a	0 a	0 a	0 a
Fomesafen + MSB (0.5% v/v)	240	11 bc	9 bc	5 b	2 b	0 a
Bentazon	1080	5 ab	5 b	3 b	2 b	1 a
Thifensulfuron-methyl + NIS (0.1% v/v) + UAN (8 L/ha)	6	18 d	18 d	12 c	8 c	4 b
Cloransulam-methyl + NIS (0.25% v/v) + UAN (2.5% v/v)	17.5	9 bc	8 bc	3 b	1 ab	1 a
Imazethapyr + NIS (0.25% v/v) + UAN (2 L/ha)	100	12 cd	12 c	6 b	6 c	3 b
HERB p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Fungicide treatment						
No fungicide		11	11	6	4	2
Pyraclostrobin/fluxapyroxad	150	11	10	5	3	1
FUNG p-value		0.6765	0.5488	0.2871	0.1454	0.1785
Interaction						
HERBxFUNG p-value		0.9968	0.9126	0.9799	0.8366	0.4697

Note: Means followed by a different lowercase letter within a column differ significantly according to a Tukey-Kramer multiple range test at $p < 0.05$. Abbreviations: DAB, days after fungicide application; FUNG, fungicide treatment; HERB, herbicide treatment; MSB, mineral oil/surfactant blend; NIS, non-ionic surfactant; UAN, 28% urea ammonium nitrate; WAB, weeks after fungicide application.

producing a 3.0 m spray width.

Soybean injury was visually estimated at 3 days after fungicide application (DAB) and 1, 2, 4, and 8 weeks after fungicide application (WAB), with 0 indicating no visible damage and 100 indicating complete soybean necrosis. Soybean vigour was assessed on percent scale relative to the non-treated control. Soybean grain yield and moisture content were measured at harvest using a Wintersteiger small plot combine equipped with a Grain Gage weighing system. Soybean yields were adjusted to 13% seed moisture content [4].

Data were analyzed using PROC GLIMMIX (SAS Ver. 9.4, SAS Institute Inc., Cary, NC). Two soybean cultivars were planted in three of the four trials conducted and were treated as subsamples for analysis. Mixed model fixed effects were herbicide treatment, fungicide treatment and their interaction; random effects were year-location combinations (environments), herbicide by fungicide by environment interaction, soybean cultivar by replicate within environment interaction, and replicate within environment. Distributions in GLIMMIX and the analysis assumptions for each variable were evaluated using information criteria, Chi-square/df ratio, studentized residual plots, as well as normal probability plots and the Shapiro-Wilk statistic from the UNIVARIATE procedure. Soybean seed moisture content at harvest and yield were converted to a percent of the non-treated control to reduce variation among environments. All variables were analyzed using a normal distribution; soybean vigour, evaluated 1 WAB, was additionally arcsine-square root transformed prior to analysis to better meet assumptions. Treatment least-square means were subjected to the Tukey-Kramer adjustment for pairwise comparisons at $p < 0.05$. The main factor level was excluded from the analysis if it had a value of zero across all environments; however, least square means could still be compared to the value zero using the p-value generated with the LSMEANS output. The main effects of herbicide and fungicide treatment were separated only if the herbicide by fungicide treatment interaction was negligible. If this interaction was non-negligible, the SLICEDIFF option was used to obtain relevant comparisons for the simple effects.

3. Results and Discussion

At 3 DAB, glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr applied POST caused 0, 11%, 5%, 18%, 9% and 12% soybean injury, respectively (**Table 1**). The injury decreased with time. At 8 WAB, glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr applied POST caused only 0, 0, 1, 4, 1 and 3% visible soybean injury, respectively (**Table 1**). Glyphosate applied POST did not cause any soybean injury at 3 DAB and 1, 2, 4 and WAB. In contrast, thifensulfuron caused the greatest soybean injury with 18%, 18%, 12%, 8% and 4% soybean injury at 3 DAB and 1, 2, 4 and WAB, respectively. The ranking of the herbicides from least to most injurious was glyphosate, bentazon, cloransulam-methyl, fomesafen, imazethapyr and thifensulfuron, at 3 DAB; generally, this same trend was observed at 1, 2, 4 and 8 WAB although differences were not always statistically significant. The application of pyraclostrobin/fluxapyroxad 2 to 3 days after the application of a herbicide applied POST did not affect soybean injury.

Soybean vigour followed the same pattern as visible injury, as herbicide induced soybean injury increased there was a concomitant decrease in soybean vigour (**Table 2**). Soybean vigour with glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr applied POST was 100%, 91%, 95%, 84%, 91% and 88%, respectively at 3 DAB (**Table 2**). Soybean vigour increased with time. Soybean vigour with glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr applied POST was 94% - 100% at 4 WAB (**Table 2**). The application of pyraclostrobin/fluxapyroxad after the application of a herbicide increase soybean vigour by 1% and 2% at 2 and 4 WAB, respectively. At 8 WAB there was an interaction between herbicide and fungicide so the simple effects are presented in **Table 3**. When no fungicide was applied, thifensulfuron and imazethapyr reduced soybean vigour 6% (**Table 3**). Interestingly, when a fungicide was applied 2 to 3 days after the herbicide appli-

Table 2. Significance of main effects and interaction for soybean vigour, relative moisture and relative yield when treated with herbicides followed by a fungicide 2 - 3 days later in trials conducted at Ridgetown, ON (2017) and Exeter, ON (2019-2020), n = 4. Means for a main effect were separated only if the interaction involving the main effect was negligible[a].

Main effects				Vigour[b]	(%)		Relative	Relative
	Rate (g·ai·ha^{-1})	3 DAB	1 WAB	2 WAB	4 WAB	8 WAB	Moisture (%)	Yield (%)
Herbicide treatment								
No herbicide		100 a	100 a	100 a	100 a	100	100	98
Glyphosate	900	100 a	100 a	100 a	100 a	100	100	98
Fomesafen + MSB (0.5% v/v)	240	91 bc	95 cd	96 c	97 bc	99	100	96
Bentazon	1080	95 b	99 b	100 a	100 a	99	100	96
Thifensulfuron-methyl + NIS (0.1% v/v) + UAN (8 L/ha)	6	84 d	93 d	97 bc	94 d	97	100	93
Cloransulam-methyl + NIS (0.25% v/v) + UAN (2.5% v/v)	17.5	91 bc	98 bc	98 b	98 ab	99	100	97
Imazethapyr + NIS (0.25% v/v) + UAN (2 L/ha)	100	88 c	94 d	97 bc	95 cd	95	99	92
HERB p-value		<0.0001	<0.0001	<0.0001	<0.0001	0.0065	0.3843	<0.0001
Fungicide treatment								
No fungicide		90	97	97 b	96 b	97	100	96
Pyraclostrobin/fluxapyroxad	150	90	96	98 a	98 a	99	100	95
FUNG p-value		0.5566	0.2359	0.0142	0.0261	0.0427	0.1314	0.5252
Interaction								
HERB × FUNG p-value		0.9897	0.6806	0.9510	0.0590	0.0176	0.5336	0.0082

Note: Means followed by a different lowercase letter within a column differ significantly according to a Tukey-Kramer multiple range test at $p < 0.05$. Abbreviations: DAB, days after fungicide application; FUNG, fungicide treatment; HERB, herbicide treatment; MSB, mineral oil/surfactant blend; NIS, non-ionic surfactant; UAN, 28% urea ammonium nitrate; WAB, weeks after fungicide application. [b]Means for vigour 2 and 4 WAB reflect one environment; vigour 8 WAB reflects two environments. Vigour values for the remaining environments were all 100.

cation there was no decrease in soybean vigour. The application of pyraclostrobin/fluxapyroxad 2 to 3 days after the application of a thifensulfuron-methyl applied POST increased soybean vigour 6% at 8 WAB. These results are similar to Swoboda and Pedersen [6] who found that soybean biomass increased 10% with foliar application of pyraclostrobin. Joshi *et al.* [25] reported that pyraclostrobin increased nitrogen fixation in soybean which can result in enhanced plant growth and increased biomass.

Soybean maturity as indicated by the seed moisture content at harvest was not affected with all treatments evaluated (**Table 2**). There was an interaction between herbicide and fungicide so the simple effects on soybean yield are presented in **Table 3**. Thifensulfuron-methyl and imazethapyr without a subsequent fungicide application reduced soybean yield 7% and 10%, respectively; the application of glyphosate, fomesafen, bentazon and cloransulam-methyl did not

Table 3. Least square means for soybean vigour 8 WAB and yield when treated with herbicides followed by a fungicide 2 - 3 days later in trials conducted at Ridgetown, ON (2017) and Exeter, ON (2019-2020), n = 4[a].

Herbicide treatment	Fungicide treatment						
	Rate ($g \cdot ai \cdot ha^{-1}$)	No fungicide			Pyraclostrobin/fluxapyroxad (150)		
Vigour 8 *WAB*[b]		%					
No herbicide		100	a		100	a	
Glyphosate	900	100	a		100	a	
Fomesafen + MSB (0.5% v/v)	240	100	a		98	a	
Bentazon	1080	99	a		99	a	
Thifensulfuron-methyl + NIS (0.1% v/v) + UAN (8 L/ha)	6	94	b	Y	100	a	Z
Cloransulam-methyl + NIS (0.25% v/v) + UAN (2.5% v/v)	17.5	98	ab		100	a	
Imazethapyr + NIS (0.25% v/v) + UAN (2 L/ha)	100	94	b		96	a	
Relative yield							
No herbicide		100	a	Z	95	ab	Y
Glyphosate	900	99	a		97	ab	
Fomesafen + MSB (0.5% v/v)	240	97	ab		95	ab	
Bentazon	1080	96	ab		96	ab	
Thifensulfuron-methyl + NIS (0.1% v/v) + UAN (8 L/ha)	6	93	bc		94	b	
Cloransulam-methyl + NIS (0.25% v/v) + UAN (2.5% v/v)	17.5	96	ab		98	a	
Imazethapyr + NIS (0.25% v/v) + UAN (2 L/ha)	100	90	c	Y	93	b	Z

Note: Means for each parameter followed by a different lowercase letter within a column (a-c) or uppercase letter within a row (Y-Z) differ significantly according to a Tukey-Kramer multiple range test at $p < 0.05$. Rows without an uppercase letter have no difference between fungicide treatments. Abbreviation: MSB, mineral oil/surfactant blend; NIS, non-ionic surfactant; UAN, 28% urea ammonium nitrate; WAB, weeks after fungicide application. [b]Means for vigour 8 WAB reflect two environments. Vigour values for the remaining environments were all 100.

reduce soybean relative to the non-treated control (**Table 3**). The application of pyraclostrobin/fluxapyroxad after the application of imazethapyr increased soybean yield 3% (**Table 3**). Pyraclostrobin/fluxapyroxad fungicide applied to the non-herbicide treated control plots reduced soybean yield 5%, the authors attribute this observation to experimental variability (**Table 3**). In other studies, Swoboda and Pedersen [6] found no seed yield advantage in absence of diseases with foliar application of strobilurin fungicides in soybean. Other researchers have also found no significant yield increases in soybean with foliar applied fungicides in absence of diseases [26] [27]. Henry *et al*. [23] reported as much as 100 $kg \cdot ha^{-1}$ seed yield increase with the application of pyraclostrobin in soybean when disease and insect pressure was minimal. Seed mass was also increased 3% with pyraclostrobin applied POST in soybean [23]. However, the authors concluded that a yield increase may not result in an increased economic benefit as input costs may be greater than the yield benefits [23]. In another study, pyraclostrobin applied POST caused no increase in soybean health or yield in the absence of diseases. Additionally, Kandel and Mueller [28] who studied soybean yield responses to various foliar fungicides in the North Central region of the United States found inconsistent yield responses with limited profitability in soybean with QoI fungicides when disease pressure was low.

4. Conclusion

This study concludes that glyphosate applied POST without and with a follow up application of pyraclostrobin/fluxapyroxad fungicide caused no visible soybean injury and there was no decrease in soybean vigour and yield. Fomesafen, bentazon, and cloransulam-methyl applied POST caused up to 11% visible injury and reduced vigour up to 9% but caused no reduction in soybean yield. The follow-up application of pyraclostrobin/fluxapyroxad fungicide after fomesafen, bentazon, and cloransulam-methyl POST application provided no reduction in soybean injury and no increase in soybean vigour and yield. Thifensulfuron-methyl and imazethapyr applied POST caused up to 18% visible injury in soybean and reduced soybean vigour up to 16%. The follow up application of pyraclostrobin/fluxapyroxad after thifensulfuron-methyl increased soybean vigour 6% and the follow up application of pyraclostrobin/fluxapyroxad after imazethapyr increased soybean yield 3%. Results indicate that pyraclostrobin/fluxapyroxad fungicide can be safely applied 2 - 3 days after POST application of glyphosate, fomesafen, bentazon, thifensulfuron-methyl, cloransulam-methyl and imazethapyr. The use of pyraclostrobin/fluxapyroxad after the application of the thifensulfuron-methyl was associated with increased vigour in soybean at 8 WAB. The use of pyraclostrobin/fluxapyroxad after the application of the imazethapyr was associated with increased soybean yield. Further studies are needed to determine the mechanisms involved.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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