

Glyphosate-Resistant Canada Fleabane Control with Three-Way Herbicide Tankmixes in Soybean

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Abstract

Eight field trials (2 in 2016, 3 in 2017, 3 in 2018) were conducted in farmers' fields with heavy infestations of GR *Conyza canadensis* (Canada fleabane, horseweed or marestalk) to evaluate glyphosate (900 g ae ha⁻¹) plus saflufenacil (25 g ai ha⁻¹), 2,4-D ester (500 g ai ha⁻¹) or paraquat (1100 g ai ha⁻¹) applied preplant (PP) as 2-way tankmixes, or in 3-way tankmixes with sulfentrazone (140 g ai ha⁻¹), flumioxazin (107 g ai ha⁻¹) or metribuzin (400 g ai ha⁻¹) for the glyphosate-resistant (GR) *C. canadensis* control in GR soybean. Glyphosate plus saflufenacil applied PP controlled GR *C. canadensis* as much as 90%. The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix provided as much as 93%, 96% and 97% control of GR *C. canadensis*, respectively. Glyphosate plus 2,4-D ester applied PP provided as much as 59% control of GR *C. canadensis*. The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix provided as much as 60%, 59% and 91% control of GR *C. canadensis*, respectively. Glyphosate plus paraquat applied PP provided as much as 85% control of GR *C. canadensis*. The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix provided as much as 88%, 89% and 98% control of GR *C. canadensis*, respectively. Density and biomass reductions of GR *C. canadensis* with herbicides evaluated followed the same pattern as weed control evaluations. GR *C. canadensis* interference reduced soybean yield 66%. Reduced GR *C. canadensis* interference with the preplant herbicides evaluated provided soybean yield similar to the weed-free control. Results from this study show that glyphosate plus saflufenacil, glyphosate plus 2,4-D ester or glyphosate plus paraquat tankmixed with metribuzin can provide effective control of GR *C. canadensis* in GR soybean.

Keywords

Biomass, Crop Injury, Density, Flumioxazin, Glyphosate, Herbicide

1. Introduction

Soybean [*Glycine max* (L.) Merr.] production is valuable to agriculture in Canada [1]. Growers in Canada annually produce nearly 5 million tonnes of soybean on 1.6 million hectares with a farm gate value of over one billion dollars [1] [2]. Nearly 70% of Canadian soybean production is grown in Ontario [1]. Effective weed management, particularly against recently evolved glyphosate-resistant (GR) weeds is rated as one of the greatest challenges faced by Ontario soybean producers.

Ontario farmers have identified GR *Conyza canadensis* (L.) Cronquist (common names include Canada fleabane, horseweed or marestail in North America) as the number one weed management issue in the province in a survey conducted in Ontario [3] [4]. *C. canadensis* is a prolific weed belonging to Asteraceae family that can produce as much as 1,000,000 seeds/plant which can subsequently be dispersed as far as 550 kilometers from the parent plant [5]. *C. canadensis* usually germinates in late summer and early fall, forming overwintering rosettes which bolt and produce numerous, elongated flowering branches in the following summer [6]. *C. canadensis* is very competitive with soybean crop and can decrease yield substantially if not adequately controlled. Bruce and Kells [7] reported 90% soybean yield reduction with 100 - 200 plant⁻² densities of *C. canadensis*. Dauer *et al.* [8] found 97% reduction in yield due to *C. canadensis* interference in soybean. Byker *et al.* [9] also reported up to 93% reduction in soybean yield when GR *C. canadensis* was not controlled. It is critical for soybean growers to adequately control this troublesome weed in their fields to avoid crop yield loss and increase crop production efficiency.

Preplant (PP) herbicide combinations that have burndown and extended residual activity are the optimal choices for GR *C. canadensis* control as this prolific weed has a long emergence pattern and most of postemergence (POST) herbicides do not effectively control this problematic weed in GR soybean [10]. Saflufenacil, a pyrimidinedione herbicide that inhibits the PPO enzyme; 2,4-D ester, a phenoxy herbicide that up-regulates gene expression; and paraquat, a bipyridilium herbicide that causes cell membrane destruction in susceptible plants are three preplant herbicides with burndown activity, but all provide relatively short residual activity against GR *C. canadensis* in soybean [11].

Earlier studies have shown that saflufenacil can control GR *C. canadensis* greater than 90% in soybean [9]. However, some studies have shown inconsistent control of *C. canadensis* with saflufenacil in soybean in Ontario [3]. Additionally, 2,4-D ester and paraquat have been reported to provide inconsistent control of GR *C. canadensis* in soybean [3] [9]. Tankmix herbicide partners with residual activity such as sulfentrazone, flumioxazin and metribuzin have the po-

tential to increase the uniformity of GR *C. canadensis* control when tankmixed with saflufenacil, 2,4-D or paraquat [3] [9] [12] [13]. Adding a third tankmix partner to these herbicides may increase efficacy and may provide the season-long residual control of GR *C. canadensis* in soybean. In addition, 3-way herbicide tankmixes can also be helpful by adding a new mode of action that is crucial for long-term sustainable weed management strategies in agricultural crops.

Few studies have collectively evaluated the 3-way tankmixes of glyphosate plus saflufenacil, 2,4-D ester or paraquat with sulfentrazone, flumioxazin or metribuzin for GR *C. canadensis* control in glyphosate-resistant soybean in Ontario. Therefore, the purpose of this study was to assess the effectiveness of glyphosate (900 g ae ha⁻¹) plus saflufenacil (25 g ai ha⁻¹), 2,4-D ester (500 g ai ha⁻¹) or paraquat (1100 g ai ha⁻¹) in 3-way tankmixes with sulfentrazone (140 g ai ha⁻¹), flumioxazin (107 g ai ha⁻¹) or metribuzin (400 g ai ha⁻¹) for GR *C. canadensis* control in GR soybean.

2. Materials and Methods

Eight experiments (2 in 2016, 3 in 2017, 3 in 2018) were conducted in farmers' fields in Ontario. Experiments were arranged as a random complete block design (RBCD) with 4 replications. Plot measurements were 2.25 m wide (3 rows of soybean) by 8 m long. Glyphosate-resistant soybean ("DKB 12-57") was seeded to a depth of approximately 4 cm, in rows spaced 0.75 m apart, at the rate of 400,000 seeds per ha⁻¹. Herbicide treatments included glyphosate (900 g ae ha⁻¹) plus saflufenacil (25 g ai ha⁻¹), 2,4-D ester (500 g ai ha⁻¹) or paraquat (1100 g ai ha⁻¹) applied alone and in combination with sulfentrazone (140 g ai ha⁻¹), flumioxazin (107 g ai ha⁻¹) or metribuzin (400 g ai ha⁻¹) as listed in **Table 1**. All saflufenacil treatments included a surfactant (Merge[®] at 1 L·ha⁻¹).

Herbicides were applied PP (up to 6 days before seeding) with a CO₂-pressurized backpack sprayer when *C. canadensis* was 10 cm in width/height. The sprayer was adjusted to deliver 200 L·ha⁻¹ of water solution at 240 kPa using a hand boom that was 1.5 m long and had four ultra-drift ULD-12-002 nozzles (Hypro[®] Ultra-Lo Drift 120-02 nozzle, 375 5th Ave. NW, New Brighton, MN 55112) spaced 0.5 m apart.

Soybean injury was assessed visually at two and four weeks after crop emerged (WAE) using a scale of 0% to 100% (0 represented no visible injury and 100 represented completely dead plants). Percent GR *C. canadensis* control was evaluated four and eight weeks after herbicide application (WAA) using a scale of 0 to 100 (0 represented no GR *C. canadensis* control and 100 represented completely dead GR *C. canadensis* plants). Density and shoot aboveground dry weight/biomass (counted and oven dried at 60 °C in a paper bag for two weeks) of GR *C. canadensis* was assessed eight WAA within two 0.25 m⁻² quadrats which were randomly placed within each plot. At harvest time, soybean plants in the two centre rows of each plot were harvested using a small plot research combine. Soybean seed yields were then adjusted to 13% seed moisture content.

Table 1. Percent glyphosate-resistant *Conyza canadensis* (Canada fleabane) control 4 and 8 WAA, fleabane density and dry biomass 8 WAA, soybean seed moisture content at harvest and seed yield for herbicide combinations applied preplant in soybean at 8 sites in southwestern Ontario from 2016 to 2018. Means followed by a different letter within a column are significantly different according to a Tukey-Kramer multiple range test at $P < 0.05$.^a

| Treatment ^b | Control | | | | Soybean | Soybean | |
|------------------------------|------------|-------------|--------|---------------------------|----------------------|----------------------------|-----------------------|
| | Rate | 4 WAA | 8 WAA | Density | Biomass | Seed Moisture ^c | Yield ^c |
| | (g ai/ha) | ————(%)———— | | (Plants m ⁻²) | (g·m ⁻²) | (%) | (T ha ⁻¹) |
| Weed-free control | | 100 | 100 | 0 a | 0 a | 12.4 a | 2.39 a |
| Weedy control | | 0 d | 0 e | 81 e | 136.5 g | 16.1 b | 0.81 b |
| Saflufenacil ^d | 25 | 90 ab | 87 abc | 4 bc | 3.1 bcd | 13.6 a | 2.25 a |
| Saflufenacil + sulfentrazone | 25 + 140 | 93 ab | 93 abc | 2 bc | 1.8 bc | 12.7 a | 2.36 a |
| Saflufenacil + flumioxazin | 25 + 107 | 96 ab | 95 abc | 2 bc | 1.5 bc | 12.5 a | 2.17 a |
| Saflufenacil + metribuzin | 25 + 400 | 96 ab | 97 ab | 1 ab | 1.5 bc | 12.3 a | 2.46 a |
| 2,4-D ester | 500 | 59 c | 53 d | 31 de | 26.9 ef | 12.9 a | 2.35 a |
| 2,4-D ester + sulfentrazone | 500 + 140 | 60 c | 56 d | 28 de | 25.8 ef | 12.7 a | 2.16 a |
| 2,4-D ester + flumioxazin | 500 + 107 | 59 c | 55 d | 30 de | 35.7 fg | 12.8 a | 1.89 a |
| 2,4-D ester + metribuzin | 500 + 400 | 91 ab | 89 abc | 3 bc | 3.3 bcd | 12.3 a | 2.56 a |
| Paraquat | 1100 | 85 b | 81 c | 8 cd | 12.3 def | 12.1 a | 2.33 a |
| Paraquat + sulfentrazone | 1100 + 140 | 88 ab | 85 abc | 5 bc | 5.5 bcde | 12.1 a | 2.47 a |
| Paraquat + flumioxazin | 1100 + 107 | 89 ab | 84 bc | 5 bc | 7.3 cdef | 12.1 a | 2.46 a |
| Paraquat + metribuzin | 1100 + 400 | 98 a | 98 a | 1 ab | 0.7 ab | 12.1 a | 2.40 a |

^a Abbreviations: WAA, weeks after herbicide application. ^b All herbicide treatments included glyphosate (900 g ae ha⁻¹). ^c Soybean seed moisture content and seed yield collected in 2017 and 2018 at 6 of 8 sites. ^d All saflufenacil treatments included non-ionic surfactant (Merge* at 1 L·ha⁻¹).

For the statistical analysis, the GLIMMIX method was used to analyze data in SAS (2016; SAS Institute Inc, Cary, NC.). In the analysis, the preplant herbicide treatment was the fixed effect and year-location combinations, replicate within the year-location and the year-location by treatment interaction were the random effects. The best distribution and associated link function for each parameter was chosen by comparing fit statistics, residual plots and the Shapiro-Wilk statistic among the potential distributions. LSMEANS were calculated by using the inverse link function, and pairwise comparisons were subjected to Tukey's adjustment before determining treatment differences at $P < 0.05$. The Gaussian distribution and identity link were used for GR *C. canadensis* control (%) 4 and 8 WAE, and yield. Additionally, the lognormal distribution and identity link were used to analyze *C. canadensis* density and biomass, while the gamma distribution and log link were used to analyze percent soybean seed moisture at harvest. The two control treatments were kept out from the analysis due to zero variance. Comparisons were still possible between the other treatments and the value zero using the LSMEANS output and differences were identified. A correction for log bias was applied to treatment means that were analyzed using the lognormal distribution.

3. Results and Discussion

3.1. GR *Conyza Canadensis* Control

Glyphosate plus saflufenacil applied PP provided as much as 90% control of GR *C. canadensis* 4 and 8 WAA (**Table 1**). The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix provided as much as 93%, 96% and 97% GR *C. canadensis* control, respectively. The control with the 3-way tankmixes was similar to glyphosate plus saflufenacil. Glyphosate plus 2,4-D ester applied PP controlled *C. canadensis* only 53% - 59% 4 and 8 WAA. The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix provided as much as 60%, 59% and 91% control of GR *C. canadensis*, respectively. Metribuzin addition to glyphosate plus 2,4-D improved GR *C. canadensis* control. Glyphosate plus paraquat applied PP provided as much as 85% GR *C. canadensis* control 4 and 8 WAA (**Table 1**). The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix provided as much as 88%, 89% and 98% GR *C. canadensis* control, respectively. Metribuzin addition to glyphosate plus paraquat improved GR *C. canadensis* control.

Byker *et al.* [9] found 88% - 100% GR *C. canadensis* control with glyphosate tankmixed with saflufenacil, 49% - 80% GR *C. canadensis* control with glyphosate tankmixed with 2,4-D ester, and 59% - 95% GR *C. canadensis* control with glyphosate tankmixed with paraquat 4 WAA in soybean. Similarly, Loux *et al.* [14] showed that a tankmix of glyphosate with saflufenacil, 2,4-D ester or metribuzin provided season-long control of GR *C. canadensis* in glyphosate-resistant soybean. Additionally, Budd *et al.* [3] reported as much as 99% GR *C. canadensis* control with glyphosate tankmixed with saflufenacil applied PP alone or in tankmix combinations with 2,4-D, metribuzin or paraquat in soybean. In contrast, other studies have found only 57% control of GR *C. canadensis* with glyphosate tankmixed with saflufenacil in glyphosate-resistant soybean [15]. Other studies have also found only 73% control of GR *C. canadensis* with glyphosate plus metribuzin in soybean [16]. Glyphosate plus saflufenacil and glyphosate plus paraquat were shown to control GR *C. canadensis* as much as 96% - 97% and 81% - 95% in cotton, respectively [17]. Keeling *et al.* [18] reported 92% - 100% control of *C. canadensis* in cotton with glyphosate tankmixed with 2,4-D ester applied PP.

In this study, adding sulfentrazone and flumioxazin to the tankmixes of glyphosate plus saflufenacil, 2,4-D ester, or paraquat did not significantly increased the control of GR *C. canadensis*. Glyphosate tankmixed with flumioxazin has been shown to minimally (28% - 46%) control GR *C. canadensis* in glyphosate-resistant soybean [9]. Norsworthy [13] and Owen *et al.* [12] reported only 50% - 63% GR *C. canadensis* control with flumioxazin tankmixes in cotton.

3.2. GR *Conyza Canadensis* Density

Glyphosate plus saflufenacil decreased density of GR *C. canadensis* 95% 8 WAA (**Table 1**). The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix

decreased GR *C. canadensis* density 98%, 98% and 99% compared to the weedy control, respectively. Density of GR *C. canadensis* was not reduced significantly with the addition of sulfentrazone, flumioxazin or metribuzin to glyphosate plus saflufenacil. Glyphosate plus 2,4-D ester decreased density of GR *C. canadensis* only 62% 8 WAA. The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix reduced GR *C. canadensis* density 65%, 63% and 96%, respectively. Adding metribuzin to glyphosate plus 2,4-D reduced GR *C. canadensis* density. Glyphosate plus paraquat decreased GR *C. canadensis* density 90% 8 WAA. Adding sulfentrazone, flumioxazin or metribuzin to the tankmix decreased GR *C. canadensis* density 94%, 94% and 99% compared to the weedy control, respectively. Adding metribuzin to glyphosate plus paraquat decreased GR *C. canadensis* density.

In another study, Budd *et al.* [3] reported 97%, 98% and 97% decrease in GR *C. canadensis* density when a 3-way tankmixes of glyphosate plus saflufenacil plus 2,4-D ester, metribuzin or paraquat were applied PP in GR soybean, respectively. However, other studies have reported only 66% decrease in GR *C. canadensis* density when glyphosate plus metribuzin were applied PP in soybean [19]. Glyphosate plus saflufenacil and glyphosate plus paraquat were shown to reduce *C. canadensis* density only 60% and 54% in cotton, respectively [17].

3.3. GR *Conyza Canadensis* Biomass

Glyphosate plus saflufenacil applied PP decreased GR *C. canadensis* biomass 98% 8 WAA (Table 1). The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix decreased GR *C. canadensis* biomass 99% 8 WAA (Table 1). GR *C. canadensis* biomass was similar with glyphosate plus saflufenacil and in the 3-way tankmixes. Glyphosate plus 2,4-D ester applied PP caused an 80% decrease in GR *C. canadensis* biomass in soybean 8 WAA (Table 1). The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix caused an 81%, 74% and 98% reduction in GR *C. canadensis* biomass in soybean 8 WAA, respectively (Table 1). Adding metribuzin to glyphosate plus 2,4-D decreased GR *C. canadensis* biomass. Glyphosate plus paraquat applied PP provided a 91% reduction in GR *C. canadensis* biomass in soybean 8 WAA (Table 1). The addition of sulfentrazone, flumioxazin or metribuzin to the tankmix provided a 96%, 95% and 99% reduction in GR *C. canadensis* biomass in soybean 8 WAA, respectively. Adding metribuzin to glyphosate plus paraquat decreased GR *C. canadensis* biomass.

In another study, Budd *et al.* [3] reported 93%, 92% and 86% decrease in biomass of GR *C. canadensis* with a 3-way tankmixes of glyphosate plus saflufenacil plus 2,4-D ester, metribuzin or paraquat in GR soybean, respectively. Additionally, Byker *et al.* [9] reported as much as 99% and 95% decrease of GR *C. canadensis* biomass with glyphosate plus saflufenacil and glyphosate plus 2,4-D ester applied PP in glyphosate-resistant soybean, respectively. However, other studies have reported only 45% reduction in biomass of GR *C. canadensis* with glyphosate plus saflufenacil in soybean [15]. Other 2-way or 3-way herbicide

tankmixes such as glyphosate + flumioxazin, glyphosate + flumioxazin + chorimuron-ethyl and glyphosate + flumioxazin + pyroxasulfone were shown to reduce GR *C. canadensis* biomass 65%, 88% and 47% in soybean, respectively [9].

3.4. Soybean Crop Responses

There was no injury in soybean at 2 and 4 WAE at all site-years with treatments evaluated, therefore data were not analyzed (data not presented).

Seed moisture of soybean was 3.7% higher in weedy control plots compared to the plots with no weeds indicating that the presence of GR *C. canadensis* delayed soybean maturity (Table 1). Seed moisture of soybean was not different between the weed-free control plots and herbicide treated plots (Table 1).

GR *C. canadensis* interference reduced soybean yield 66% (Table 1). This finding is consistent with another study that has shown 73% reduction in yield due to GR *C. canadensis* interference in GR soybean [3]. However, other studies have shown as much as 82% - 97% reduction in yield from GR *C. canadensis* interference in GR soybean [8] [9] [19].

Reduced GR *C. canadensis* interference with the preplant herbicides evaluated provided soybean yield similar to the weed-free control. Budd *et al.* [3] also reported no soybean seed yield loss with a 3-way tankmixes of glyphosate plus saflufenacil plus 2,4-D ester, metribuzin or paraquat.

4. Conclusion

Glyphosate plus saflufenacil applied PP has the potential to adequately control GR *C. canadensis* in soybean. Adding sulfentrazone, flumioxazin or metribuzin to glyphosate plus saflufenacil did not significantly enhance *C. canadensis* control. Glyphosate plus paraquat applied PP has the potential to adequately control GR *C. canadensis* in soybean. Adding metribuzin to glyphosate plus paraquat enhanced *C. canadensis* control and reduced density and biomass of *C. canadensis*, however, there was no benefit of adding sulfentrazone and flumioxazin to this tankmix for the control of *C. canadensis*. Glyphosate plus 2,4-D ester applied PP provided poor control of GR *C. canadensis*. The addition of sulfentrazone or flumioxazin to glyphosate plus 2,4-D did not enhance the control of *C. canadensis* and there was no decrease in density and biomass of GR *C. canadensis*. In contrast, adding metribuzin to glyphosate plus 2,4-D increased *C. canadensis* control and decreased density and biomass of GR *C. canadensis*. This study concludes that metribuzin tankmixed with glyphosate plus saflufenacil, glyphosate plus 2,4-D ester, or glyphosate plus paraquat can be used for consistent season-long control of GR *C. canadensis* in GR soybean. The use of a 3-way tankmix can be helpful in decreasing the natural selection of herbicide-resistant *C. canadensis*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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