Glyphosate-resistant horseweed control in glyphosate/glufosinate/2,4-D-resistant soybean with one- and two-pass herbicide programs

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Abstract
Glyphosate-resistant (GR) biotypes of horseweed were first confirmed in southern Ontario in 2010 and have spread across southern Ontario. A total of four field experiments were conducted between 2021 and 2022 to determine GR horseweed control with one- and two-pass herbicide programs in glyphosate/glufosinate/2,4-D-resistant (GG2R) soybean. 2,4-D choline/glyphosate DMA, halauxifen-methyl, and saflufenacil applied preplant (PP) controlled GR horseweed by 59%, 72%, and 78% 8 wk after postemergence (POST) application (WAA-POST); there was no improvement of GR horseweed control when 2,4-D choline/glyphosate DMA was added to saflufenacil; in contrast, there was improved GR horseweed control when saflufenacil was added to 2,4-D choline/glyphosate DMA. Glufosinate and 2,4-D choline/glyphosate DMA applied POST controlled glyphosate-resistant horseweed by 71% and 86%, respectively, 8 WAA-POST. Two-pass herbicide programs of a PP followed by POST application provided greater GR horseweed control than a PP or POST herbicide applied alone. Glufosinate or 2,4-D choline/glyphosate DMA applied POST following 2,4-D choline/glyphosate DMA or halauxifen-methyl applied PP improved GR horseweed control by 29% to 38% and 24%, respectively at 8 WAA-POST. The application of 2,4-D choline/glyphosate DMA applied POST following saflufenacil applied PP improved control by 20% 8 WAA-POST; there was no improvement of GR horseweed control when saflufenacil was applied POST following saflufenacil applied PP or when either POST herbicide was applied following saflufenacil + 2,4-D choline/glyphosate DMA applied PP. When used in a two-pass program, 2,4-D choline/glyphosate DMA POST provided 2% to 3% greater control of GR horseweed than glufosinate.

Introduction
Horseweed is a common, nuisance weed for Ontario soybean producers. Native to North America, horseweed belongs to the Asteraceae family with a winter or summer annual growth habit (Weaver 2001). The fall-emerging cohort has a competitive advantage over the spring-emerging cohort because it is in a more advanced growth stage relative to the spring-emerging summer annual cohort (Buhler and Owen 1997). However, the spring-emerging cohort may escape control from preplant (PP) nonresidual herbicide applications by emerging after herbicide applications (Buhler and Owen 1997). A ruderal weed that germinates best when seeds are on the soil surface, horseweed has a high capability to thrive under reduced-, strip-, and no-till cropping systems (Nandula et al. 2006; Weaver 2001).

One horseweed plant can produce up to 500,000 seeds with the potential to germinate immediately after seed release (Davis et al. 2009; Weaver 2001). Horseweed plants that grow taller than the soybean canopy are more competitive with increased seed production compared with plants that grow below the canopy (Davis and Johnson 2008). Seeds are distributed by wind, commonly landing within 100 m of the parent plant, but have the potential to move hundreds of kilometers if the seed enters the planetary boundary layer (Dauer et al. 2006; Shields et al. 2006). Horseweed interference can cause substantial yield losses in soybean, especially fall-emerged cohorts at high densities. Uncontrolled horseweed can decrease soybean seed yield by up to 93% (Byker et al. 2013a). The burial of horseweed seeds with tillage is an effective method of reducing horseweed populations (Nandula et al. 2006). Tillage is not an option in crop production systems that use no-till methods; consequently, herbicides are relied upon for horseweed control.

The introduction of transgenic soybean that is resistant to glyphosate played an integral role in the expansion of minimum tillage (Carpenter and Gianessi 1999). The swift uptake in the use of glyphosate-resistant (GR) crops accompanied by an exponential increase in the use of...
glyphosate in agricultural lands has placed intense selection pressure on the evolution of GR weed biotypes. Horseweed was the first annual dicotyledonous plant to evolve resistance to glyphosate in a GR crop (VanGessel 2001). The initial confirmation of GR horseweed in Ontario was from a field located in Essex County in 2010 (Byker et al. 2013b). Since 2010, GR horseweed populations have been confirmed in 30 Ontario counties up to 750 km from the original point of discovery (Budd et al. 2018). GR horseweed biotypes have little to no fitness penalty relative to susceptible biotypes (Kruger et al. 2010). After an application of glyphosate, some GR horseweed biotypes have yellow discoloration in the growing point and produce new branches from basal axillary nodes; these plants produce viable seeds with resistant offspring (Dinelli et al. 2006), whereas other GR biotypes exhibit no symptomology following glyphosate application. The use of diverse crop and weed management practices are recommended to curb GR horseweed populations and reduce weed seed return (Budd et al. 2018).

One response to the evolution of GR weeds is the expansion of herbicide-resistant (HR) crops with multiple herbicide-resistance traits. These HR crops can delay the further evolution of GR weeds by allowing multiple herbicide modes of action to be applied to the crop (Duke 2014). Producers have rapidly adopted HR technologies because of improved weed control, minimized crop injury, reduced complexity of weed management, and decreased environmental impact of herbicides (Mall et al. 2019).

Glyphosate/glufosinate/2,4-D choline-resistant (GG2R) soybean (E3 soybean TM) is now commercially available in Ontario. These cultivars possess the transgene [aryloxyalkanoate dioxygenase-12 (AAD-12)] that confers resistance to 2,4-D (Wright et al. 2010). AAD-12 has been inserted into soybean cultivars, which cleaves 2,4-D into nontoxic compounds dichlorophenol and glyoxylate (Wright et al. 2010). AAD-12-derived resistance when stacked with other HR traits provides additional control options for GR weed biotypes (Craigmytle et al. 2013; Wright et al. 2010). GG2R soybean also expresses the phosphinothricin acetyltransferase (PAT) and CP4 EPSPS genes that confer resistance to glufosinate and glyphosate, respectively (Duke and Powles 2008; Takano and Dayan 2020).

The effectiveness of numerous PP herbicides for the control of GR horseweed in soybean has been explored. Horseweed control with synthetic auxin herbicides is influenced by herbicide rate and plant size at herbicide spray time (Byker et al. 2013a). 2,4-D choline/glufosinate DMA (1,720 g ai ha−1) provides inconsistent GR horseweed control (Ford et al. 2014a). Halauxifen-methyl (5 g ai ha−1) applied PP suppressed GR horseweed by 72% in a study conducted in Ontario (Quinn et al. 2021). Saflufenacil (25 g ai ha−1) and saflufenacil (25 g ai ha−1) plus 2,4-D choline/glufosate DMA (1,720 g ai ha−1) were applied when GR horseweed grew to an average height or diameter of 10 cm (Table 2). POST herbicide treatments [glufosinate (500 g ai ha−1) and 2,4-D choline/glufosate DMA (1,720 g ai ha−1)] were applied as soon as GR horseweed plants reached 10 cm in height in any PP herbicide treatment plot; one POST application was made. All POST herbicide applications were made after 9:00 AM and before 11:00 AM to reduce the time-of-day at application effect on glufosinate efficacy (Martinson et al. 2005; Montgomery et al. 2017; Takano and Dayan 2020).

Visual assessments of crop injury were evaluated 2 wk after soybean emergence (WAE), 4 wk after the PP herbicide was applied (WAA-PP), and 1 and 4 wk after the POST herbicide application (WAA-POST) on an evaluation scale of 0 to 100, where 0 indicates no injury and 100 represents complete soybean necrosis. Visible GR horseweed control assessments (an estimation of biomass decrease compared to the weedy control in each replicate) were completed on a 0 to 100 scale, where 0 indicates similar biomass to the control and 100 represents complete biomass elimination, 2 and 4 WAA-PP, and 4 and 8 WAA-POST. GR horseweed density and dry weight (above-ground biomass) were determined 4 WAA-POST from two 0.25-m2 quadrats placed arbitrarily within all plots within the treated area. GR horseweed plants inside each quadrat were counted, cut at the soil surface, and placed in labeled paper bags, dried to constant moisture, and the dry weight recorded. Upon crop maturity, the center two soybean rows were combined with a small-plot combine; soybean seed moisture content and weight were recorded. Soybean yield was corrected to 13.0% seed moisture content prior to statistical analysis.

The aim of this research was to evaluate one- and two-pass herbicide programs and to compare 2,4-D choline/glyphosate DMA versus glufosinate applied POST in two-pass weed control strategies to effectively control GR horseweed in GG2R soybean.

Methods and Materials

A total of four field trials were carried out in commercial no-tillage soybean fields in Ontario, Canada, over a 2-yr period (2021, 2022). In 2021, one trial was located near Kintyre (42.57°N, 81.77°W), and another near Bothwell (42.62°N, 81.91°W); in 2022, one trial was near Kintyre, and the second near Duart (42.51°N, 81.73°W) (Table 1). Each field location contained GR horseweed biotypes. The treatments and associated information on the herbicides evaluated in this study are included in Tables 2 through 4. The PP herbicide application timing is referred to as Application A and the POST herbicide application timing is referred to as Application B. The treatments were arranged in a randomized complete block design containing four replications. Each plot was 2.25 m wide (containing three rows of soybean spaced 75 cm apart) by 8 m in length. GG2R soybean [Brevant seeds cultivar ‘B061FE’ (Corteva Agriscience, Calgary, AB)] was planted at a rate of approximately 420,000 seeds ha−1 to a depth of approximately 4.0 cm. The herbicide treatments were applied using a CO2-pressurized backpack sprayer set to deliver a spray volume of 200 L ha−1 at 240 kPa using four ULD 11002 spray nozzles (Pentair, New Brighton, MN, USA) at a 0.5-m spacing delivering a 2-m spray pattern. PP herbicide treatments [2,4-D choline/glufosinate DMA (1,720 g ae ha−1), halauxifen-methyl (5 g ai ha−1), saflufenacil (25 g ai ha−1), and saflufenacil (25 g ai ha−1) plus 2,4-D choline/glufosate DMA (1,720 g ae ha−1)] were applied when GR horseweed grew to an average height or diameter of 10 cm (Table 2). POST herbicide treatments [glufosinate (500 g ai ha−1) and 2,4-D choline/glufosate DMA (1,720 g ae ha−1)] were applied as soon as GR horseweed plants reached 10 cm in height in any PP herbicide treatment plot; one POST application was made. All POST herbicide applications were made after 9:00 AM and before 11:00 AM to reduce the time-of-day at application effect on glufosinate efficacy (Martinson et al. 2005; Montgomery et al. 2017; Takano and Dayan 2020).

Statistical Analysis

All data were subjected to mixed model variance analysis using the GLIMMIX procedure in SAS software (version 9.4; SAS Institute.
Injury. The PP and POST herbicides evaluated caused minimal soybean injury (<5%); data not presented.

**Results and Discussion**

**Soybean Injury**

The PP and POST herbicides evaluated caused minimal soybean injury (<5%); data not presented.

**Glyphosate-Resistant Horseweed Control**

The control varied among PP herbicide treatments. Applied PP, 2,4-D choline/glyphosate DMA and haloxifen-methyl controlled GR horseweed by 74% and 81%, respectively 2 WAA-PP, which was less than saflufenacil (97%); there was no improvement of GR horseweed control with the addition of 2,4-D choline/glyphosate DMA to saflufenacil (99%; Table 3). At 4 WAA-PP, both saflufenacil and saflufenacil + 2,4-D choline/glyphosate DMA provided greater control of GR horseweed compared to the 2,4-D choline/glyphosate DMA applied PP; control with haloxifen-methyl was intermediate and comparable to all (Table 3). Ford et al. (2014b) observed that 2,4-D choline/glyphosate DMA applied PP controlled GR horseweed by 64% to 80%, which is similar to this study. Byker et al. (2013c) also observed 88% to 100% control of GR horseweed 4 WAA with saflufenacil applied PP.

When applied POST, following no PP herbicide, glufosinate and 2,4-D choline/glyphosate DMA controlled GR horseweed by 77% and 84%, respectively, 4 WAA-POST (Table 4). The one-pass PP herbicides controlled GR horseweed by 63% to 89%; saflufenacil + 2,4-D choline/glyphosate DMA provided greater control of GR horseweed compared to 2,4-D choline/glyphosate DMA; haloxifen-methyl and saflufenacil provided intermediate control and were similar to all. There was no enhancement of GR horseweed control with the addition of 2,4-D choline/glyphosate DMA to saflufenacil. The two-pass programs of a PP herbicide fb glufosinate or 2,4-D choline/glyphosate DMA applied POST controlled GR horseweed similarly at 91% to 99%;
control was improved with all two-pass programs with the exception of saflufenacil + 2,4-D choline/glyphosate DMA applied PP fb glufosinate applied POST 4 WAA-POST. Based on nonorthogonal contrasts a two-pass herbicide program provided greater GR horseweed control compared to a single PP or POST herbicide application. An application of a PP fb a POST herbicide provided 19% and 17% greater control of GR horseweed compared to a single PP or POST herbicide application, respectively, at 4 WAA-POST. The single PP or POST herbicides evaluated provided similar control of GR horseweed. When applied in a two-pass system, 2,4-D choline/glyphosate DMA POST controlled GR horseweed 2% more than glufosinate applied POST. A similar pattern was observed for GR horseweed control 8 WAA-POST. The PP herbicides controlled GR horseweed by 59% to 89% 8 WAA-POST; saflufenacil + 2,4-D choline/glyphosate DMA provided greater control of GR horseweed compared to 2,4-D choline/glyphosate DMA; halaxifien-methyl and saflufenacil applied PP provided intermediate control and were similar to all. The two-pass programs of a PP herbicide fb glufosinate or 2,4-D choline/glyphosate DMA applied POST, controlled GR horseweed similarly at 88% to 98%. GR horseweed control was increased by 29 and 38 percentage points when 2,4-D choline/glyphosate DMA applied PP was followed by glufosinate or 2,4-D choline/glyphosate DMA applied POST, respectively. Similarly, Ford et al. (2014a) reported 100% control of GR horseweed 4 and 8 WAA-POST with two passes of 2,4-D choline/glyphosate DMA applied to field corn; in that study, one PP application of 2,4-D choline/glyphosate DMA resulted in inconsistent GR horseweed control; adding a second herbicide application improved consistency of control (Ford et al. 2014a). GR horseweed control was increased by 24 percentage points when halaxifien-methyl applied PP was fb a POST-applied herbicide. GR horseweed control was increased by 20 percentage points when saflufenacil applied PP was fb 2,4-D choline/glyphosate DMA; there was no improvement in control when saflufenacil applied PP was fb glufosinate applied POST. There was no enhancement of GR horseweed control with saflufenacil + 2,4-D choline/glyphosate DMA applied POST followed by either POST-applied herbicide. The level of GR horseweed control at 8 WAA-POST in this study is consistent with findings by Quinn et al. (2021) who reported 72% GR horseweed control with halaxifien-methyl applied PP. Based on nonorthogonal contrasts a two-pass herbicide program provided greater control of GR horseweed compared to a single PP or POST herbicide application. An application of a PP fb a POST herbicide provided 20% and 17% greater control of GR horseweed compared to a single PP or POST herbicide application, respectively, at 8 WAA-POST. The single PP or POST herbicides evaluated provided similar control of GR horseweed. When applied in a two-pass system, 2,4-D choline/glyphosate DMA POST caused greater control of GR horseweed than glufosinate applied POST.

### Gly savings

Glufosinate and 2,4-D choline/glyphosate DMA applied POST and 2,4-D choline/glyphosate DMA, halaxifien-methyl, and saflufenacil applied PP resulted in GR horseweed density that was similar to the nontreated control; in contrast, saflufenacil + 2,4-D choline/glyphosate DMA applied PP decreased GR horseweed density by 96%. All the two-pass herbicide applications reduced GR horseweed density from 87% to 95%, with the exception of saflufenacil + 2,4-D choline/glyphosate DMA applied PP fb 2,4-D choline/glyphosate DMA applied POST. Based on nonorthogonal contrasts a two-pass herbicide program reduced GR horseweed density more than a single PP or POST application. There was no difference in GR horseweed density among a single PP or POST application. When applied in a two-pass system, a PP-applied herbicide fb glufosinate or 2,4-D choline/glyphosate DMA applied POST reduced GR horseweed density similarly.

### All herbicides

All herbicide applications decreased GR horseweed biomass ≥75% compared to the nontreated weedy control. Glufosinate and 2,4-D choline/glyphosate DMA POST application reduced GR horseweed biomass by 92% and 75%, respectively. 2,4-D choline/glyphosate DMA, halaxifien-methyl, saflufenacil, and saflufenacil + 2,4-D choline/glyphosate DMA applied PP decreased GR horseweed biomass by 89%, 95%, 87%, and 97%, respectively. All two-pass weed control programs decreased GR horseweed biomass similarly at 98% to 100%. Based on nonorthogonal contrasts a two-pass herbicide program reduced GR horseweed biomass more than a single PP or POST application. There was no difference in GR horseweed biomass with a single PP or POST application. When applied in a two-pass system, a PP herbicide fb glufosinate or 2,4-D choline/glyphosate DMA applied POST reduced GR horseweed biomass similarly.

### Soybean Yield

Interference from GR horseweed reduced soybean seed yield by up to 53% in this study (the greatest yielding treatment compared to the nontreated weedy control; Table 4). Reduced GR horseweed interference with all herbicide treatments evaluated resulted in greater soybean yield than the nontreated weedy control. Two passes of 2,4-D choline/glyphosate DMA, a single PP application of saflufenacil + 2,4-D choline/glyphosate DMA, and saflufenacil + 2,4-D choline/glyphosate DMA fb glufosinate, resulted in greater soybean yield than a single POST application of 2,4-D choline/glyphosate DMA. Based on nonorthogonal contrasts, the application of a POST herbicide following a PP herbicide resulted in greater soybean yield compared to a single POST application. One-pass programs of a PP herbicide provided greater soybean yield than a POST application.

### Summary

In summary, two-pass weed control strategies provide increased GR horseweed control compared to a single PP or POST herbicide application in GG2R soybean. When used in a two-pass program, 2,4-D choline/glyphosate DMA applied POST gives greater control of GR horseweed than glufosinate applied POST. Full-season control of GR horseweed is advised to reduce weed seed return and slow the spread of GR horseweed. HR crop technologies play a role in diversifying chemical weed control options by allowing alternate modes of action to be applied.
POST. Growers should also implement a diverse, integrated weed management program to increase the longevity of these valuable weed management tools.

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