Control of glyphosate-resistant Canada Fleabane in Ontario with multiple effective sites-of-action in glyphosate/dicamba-resistant soybean

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Control of glyphosate-resistant Canada Fleabane in Ontario with multiple effective sites-of-action in glyphosate/dicamba-resistant soybean

Brittany K. Hedges, Nader Soltani, Darren E. Robinson, David C. Hooker and Peter H. Sikkema

Abstract: Canada fleabane is a winter or summer annual weed that is found throughout North America. Fall-emerged Canada fleabane can fix carbon early in the growing season, giving it a competitive advantage over nearby crop and weed species. Glyphosate-resistant (GR) Canada fleabane was originally found in one county in Ontario, Canada in 2010 and had spread to at least 29 additional counties within the province by 2016. Previous research with several pre-plant (PP) herbicides resulted in variable control of GR Canada fleabane in soybean. The objective of this study was to evaluate the efficacy of glyphosate/dicamba (1800 g ae ha\(^{-1}\)) alone or with the addition of a second effective site-of-action for the control of GR Canada fleabane in glyphosate/dicamba-resistant. At 4 WAA, glyphosate/dicamba plus saflufenacil, saflufenacil/dimethenamid-P, saflufenacil/imazethapyr, or paraquat controlled GR Canada fleabane 97, 96, 97 and 98%, respectively. All herbicide treatments decreased Canada fleabane density and biomass by 93-99%. When choosing herbicide programs, it is important to consider the use of multiple sites-of-action to decrease the selection pressure for the evolution of herbicide-resistant Canada fleabane. Treatments containing saflufenacil,

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Abbreviations: GR, glyphosate-resistant; POST, postemergence; PP, pre-plant; PRE, preemergence; WAA, weeks after application; WAE, weeks after emergence.
saflufenacil/dimethenamid-P or saflufenacil with the addition of glyphosate/dicamba are recommended for the control of glyphosate-resistant Canada fleabane.

**Key words:** Canada fleabane, horseweed, preemergence, glyphosate resistance, soybean, dicamba

**Introduction**

Canada fleabane (Conyza canadensis (L.) Cronq.), also known as horseweed or marestail, is a member of the Asteraceae family and is native to North America (Weaver et al. 2001; Loux et al. 2006). Canada fleabane is a winter or summer annual weed species that produces seeds capable of germinating in the fall after maturation and seed shed due to the absence of seed dormancy (Buhler and Owen 1997). Tozzi and Van Acker (2014) reported that the majority of Canada fleabane emerges in Ontario during the last two weeks of May and a three-week period from the last week in August until the second week of September. Biotypes of Canada fleabane in Ontario can germinate at temperatures as low as 8°C, while biotypes from different areas around the world germinate at higher temperatures (Tozzi et al. 2013). Fall-emerging Canada fleabane is photosynthetically active at low temperature; making them more competitive with annual crops seeded in the spring for nutrients, space, water, and light (Main et al. 2006). Glyphosate-resistant (GR) Canada fleabane interference at a density of 167 to 750 plants m\(^{-1}\) prior to soybean emergence caused soybean yield losses of up to 93% in Ontario when no herbicide was applied (Byker et al. 2013).

Dispersal of Canada fleabane seed is facilitated by a 2-3 mm pappus, which can efficiently disperse seed hundreds of metres from the source (Royer and Dickenson 1999). Shield et al.
(2006) found Canada fleabane seeds in the Planetary Boundary Layer, which is evidence that seed may be dispersed hundreds of kilometers from the parent plant. Canada fleabane can produce up to 200,000 seeds per plant when grown in an environment free from competition from neighbouring plants, while Canada fleabane escapes in soybean can produce up to 72,000 seeds per plant (Weaver 2001; Davis and Johnson 2008).

Herbicide resistance is a growing issue around the world with 487 unique cases as of 2017 (Heap 2018). Currently, Canada fleabane populations are resistant to at least one of five known herbicide site-of-actions: Group 2 [acetolactate synthase (ALS) inhibitors], Group 5 and Group 7 [photosystem II (PSII) inhibitors at two different sites-of-action], Group 9 [5-enolpyruvoylshikimate-3-phosphate synthase (EPSPS) inhibitors] and Group 22 [photosystem I electron diverters (PSI) inhibitors] (Heap 2018). Multiple resistance to herbicides with two different sites-of-action have been confirmed in several Canada fleabane biotypes around the world (Heap 2018). In Canada, glyphosate-resistant Canada fleabane was first observed in Essex County, Ontario in 2010 and has since been found in 29 additional counties in Ontario from the most southern to the most eastern (Budd et al. 2016a).

Integrated weed management practices are required to reduce the selection pressure for herbicide-resistant weeds; some options include the use of narrow rows, herbicide tankmixes, strategic tillage, cover crops and equipment modifications (Upadhyaya and Blackshaw 2007; Walsh et al. 2013). Herbicides applied together with different modes-of-action delay the evolution of herbicide resistance longer than sequential herbicide applications with different modes-of-action (Beckie et al. 2009).

Previous research found that residual herbicides applied in the spring provide better control of spring-emerging Canada fleabane than herbicides applied in the fall (Loux et al. 2006; Davis et
al. 2007). Unacceptable control of Canada fleabane was observed with paraquat (560 g ai ha⁻¹), metribuzin (420 g ai ha⁻¹), saflufenacil/imazethapyr + glyphosate (100 + 900 g ai/ae ha⁻¹), S-metolachlor/metribuzin + glyphosate (1943 + 900 g ai/ae ha⁻¹) and saflufenacil/dimethenamid-P + glyphosate (245 + 900 g ai/ae ha⁻¹), therefore, there are very few options for the control of GR Canada fleabane (Bruce and Kells 1990; Soltani et al. 2017). Other herbicides or herbicide combinations should be evaluated for the control of GR Canada fleabane.

The use of dicamba as an effective site-of-action for weed management in glyphosate/dicamba-resistant soybean (Roundup Ready 2 Xtend® soybean) is one transgenic option growers can use to control glyphosate-resistant weeds. Other transgenic options include: glufosinate-resistant crops and glyphosate/2,4-D resistant corn; with more herbicide-resistant hybrids/cultivars projected to come to market within the next decade. Dicamba is a growth regulator herbicide that previously had to be applied three to four weeks before soybean planting to minimize soybean injury and yield loss (Thompson et al. 2007). Glyphosate/dicamba-resistant soybean contains two transgenes that code for resistance to glyphosate and dicamba, allowing both herbicides to be applied pre-plant (PP), pre-emergence (PRE) and postemergence (POST) of the crop.

The objective of this study was to evaluate the efficacy of glyphosate/dicamba with the addition of a second effective site-of-action for the control of GR Canada fleabane. This research is important to delay the selection of resistance to additional effective herbicide sites-of-action, sustaining the efficacy of current herbicide sites-of-action for commercial growers in the future.

**Materials and Methods**
This study was conducted at four locations in 2016 and three locations in 2017 for a total of seven site-years. Field trials were conducted in farmer’s fields which had a heavy, consistent infestation of GR Canada fleabane throughout the trial location. Locations were near Harrow, Ontario (42.035582, -82.918173), in 2016 and 2017, near Mull, Ontario (42.401671, -81.991098) in 2016 and 2017, two locations were near Blenheim, Ontario (42.335561, -81.997442) in 2016 and one near Thamesville, Ontario (42.551722, -81.977180) in 2017. All experiments consisted of 10 treatments arranged in a random complete block design (RBCD) with 4 replications. The treatment list included a weedy control, weed-free control and 8 herbicide treatments (Table 1). All herbicides were applied PP in spring, when Canada fleabane plants were approximately 10 cm in diameter or height. The weed-free control received a PP application of a non-residual herbicide: 2:1 premix formulation of glyphosate and dicamba (1800 g ae ha\(^{-1}\)) plus two residual herbicides: saflufenacil (25 g ai ha\(^{-1}\)) + metribuzin (400 g ai ha\(^{-1}\)). Any weed escapes in the weed-free control were removed by hand weeding. One in-season cover spray per site of glyphosate (450 g ae ha\(^{-1}\)) was applied to the entire experimental area to remove other weed species. Soybean was seeded 0 to 6 days after herbicide application with a three row no-till planter at approximately 400,000 seeds per ha\(^{-1}\) to a depth of 4 cm. Soybean cultivars DKB14-41 and DKB10-01 (Monsanto Canada Inc., Winnipeg, MB), were seeded in 2016 and 2017, respectively. Plots were 2.25 wide (3 soybean rows spaced 0.75 m apart) by 8 m in length with a 2 m walkway between blocks.

Soybean injury was evaluated 2 and 4 weeks after emergence (WAE) on a scale of 0 to 100%, where 0 was no visible injury and 100 was plant death compared to the weedy control. Weed control was evaluated visually at 2, 4, 8 and 12 weeks after application (WAA) on a scale of 0 to 100, where 0 represented no control and 100 was complete GR Canada fleabane control.
fleabane density and biomass was determined 4 WAA by counting and cutting the Canada fleabane plants within two randomly placed, 0.25 m$^2$ quadrats per plot in the center row. To determine plant biomass, plants were cut at the soil surface, placed in a paper bag, dried in a kiln at 60°C for two weeks before the weight was recorded. Soybean was harvested by hand in 2016 by cutting two, 1-m subsamples in a plot and threshing with an Almaco thresher (Almaco, Nevada, IA). In 2017, an Almaco small-plot combine was used to harvest two rows of soybean per plot. Seed moisture was measured at harvest and yields were adjusted to 14.5% moisture before analysis.

Data were analyzed as a RBCD using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC). Herbicide treatment was the fixed effect, and environment (year x location) and block were the random effects. PROC UNIVARIATE was utilized to assess residuals for normality, homogeneity and errors independent from one another (Bowley 2015). All environments were combined for analysis. The weedy and weed-free controls were not included in the analysis of Canada fleabane control, and the weed-free control was not included in the analysis of Canada fleabane density and biomass. Control data at 2 WAA was fit to a beta distribution; the cumulative complementary log-log link was used because the dataset was large. Canada fleabane control at 4, 8 and 12 WAA were fit to a normal distribution and identity link. Data from 2 WAA were backtransformed in SAS using the ilink option. The use of different distributions enabled use of the best-fitting models for analysis (Bowley 2015). Density and biomass data were analyzed using a lognormal distribution with the identity link and backtransformed within SAS. Yield data were analyzed using a normal distribution. All data were analyzed with a multiple comparison Fisher protected LSD and Tukey-Kramer adjustment, with an accepted significance value of p<0.05.
Results and Discussion

Injury

At 2 and 4 WAE, soybean injury was $\leq 10\%$ at all site-years (data not presented).

Canada fleabane control

At 2 WAA, glyphosate/dicamba with the addition of either saflufenacil, saflufenacil/dimethenamid-$P$, saflufenacil/imazethapyr or paraquat were the most efficacious herbicide applications and controlled GR Canada fleabane 97 to 98\% (Table 1). Glyphosate/dicamba applied alone and glyphosate/dicamba + 2,4-D ester were the least efficacious options with GR Canada fleabane control of 54\%.

At 4 WAA, the most efficacious herbicide tankmixes were glyphosate/dicamba with the addition of saflufenacil, saflufenacil/dimethenamid-$P$, saflufenacil/imazethapyr or paraquat, which provided 97, 96, 97, and 98\% GR Canada fleabane control, respectively (Table 1). Glyphosate/dicamba controlled GR Canada fleabane 87\%; there was no increase in control with the addition of 2,4-D ester, metribuzin or metribuzin + chlorimuron. In this study, glyphosate/dicamba + 2,4-D ester was applied PP when GR fleabane plants were up to 10 cm in height and provided 90\% of GR Canada fleabane. In contrast, Kruger et al. (2010) reported 97\% control of 7 to 15 cm tall Canada fleabane after an application of 2,4-D ester (560 g ae ha$^{-1}$).

Previous research by Waggoner et al. (2011) observed 96 and 65\% control of GR Canada fleabane with a PP application of saflufenacil (25 g ai ha$^{-1}$) at 1 and 4 WAA, respectively. In the same study, paraquat (702 g ai ha$^{-1}$) applied PP provided 84 and 70\% control of GR Canada fleabane, 1 and 4 WAA, respectively. Less control was observed 4 WAA with both treatments,
which may be due to poor coverage with saflufenacil and paraquat which are both contact herbicides.

At 8 and 12 WAA, glyphosate/dicamba controlled GR Canada fleabane 94 and 92%, respectively (Table 1). There was no improvement in GR Canada fleabane control with any of the tankmixes evaluated. At 8 WAA, Soltani et al. (2017) observed 70, 77, 68, 61, 91, and 61% control of Canada fleabane with saflufenacil, saflufenacil/dimethenamid-P, saflufenacil/imazethapyr, 2,4-D ester, metribuzin and chlorimuron-ethyl + metribuzin, respectively. In contrast, all treatments in this study controlled GR Canada fleabane 91 to 98%, 8 WAA. The addition of glyphosate/dicamba to residual herbicides in this study improved control of GR Canada fleabane compared to when used alone in previous research (Soltani et al. 2017). Glyphosate/dicamba + metribuzin controlled GR Canada fleabane 92%, which was similar to metribuzin applied alone in previous research (Soltani et al. 2017). Moseley and Hagood (1990) observed similar Canada fleabane control to this study with a PP application chlorimuron + metribuzin (90%), however less control with metribuzin alone (78%).

In previous research by Budd et al. (2016b), glyphosate (900 g ae ha\(^{-1}\)) + saflufenacil (25 g ai ha\(^{-1}\)) controlled GR Canada fleabane by 99% at 4 WAA. In this study, glyphosate/dicamba controlled GR Canada fleabane 54 and 87% at 2 and 4 WAA, respectively, which indicates that dicamba is a slower acting herbicide than saflufenacil. In the study by Budd et al. (2016b), glyphosate (900 g ae ha\(^{-1}\)) + saflufenacil (25 g ai ha\(^{-1}\)) controlled GR Canada fleabane 88% in soybean at 8 WAA, while in this study, glyphosate/dicamba + saflufenacil controlled GR Canada fleabane 94%, 8 WAA. While statistical inferences cannot be made to research previously conducted, there is higher control when glyphosate/dicamba was added to saflufenacil in this study compared to glyphosate + saflufenacil in research conducted by Budd et al. (2016).
Therefore, there might be an increase in control along with the addition of dicamba to glyphosate + saflufenacil plus the benefit of the addition of a second effective mode-of-action, which is important for resistance management of GR weeds. Budd et al. (2016b) also found the control of GR Canada fleabane with glyphosate (900 g ae ha\(^{-1}\)) + saflufenacil (25 g ai ha\(^{-1}\)) was improved with the addition of dicamba at 600 g ae ha\(^{-1}\) but there was no improvement when dicamba was added at 300 g ae ha\(^{-1}\). In this study, glyphosate/dicamba was applied at 1800 g ae ha\(^{-1}\) (2:1 ratio); this high rate of glyphosate/dicamba may be beneficial based on previous research. Additionally, Budd et al. (2016b) showed that glyphosate with the addition of saflufenacil, saflufenacil/dimethenamid-P, saflufenacil/imazethapyr, 2,4-D ester, metribuzin or chlorimuron-ethyl + metribuzin applied at the same rates as in this study controlled GR Canada fleabane 74, 79, 74, 68, 92 and 67% respectively, 4 WAA. In this study, tank mixing glyphosate/dicamba with saflufenacil, saflufenacil/dimethenamid-P, saflufenacil/imazethapyr, 2,4-D ester, metribuzin or chlorimuron-ethyl + metribuzin controlled GR Canada fleabane 97, 96, 97, 90, 88 and 85% respectively, 4 WAA. Therefore there may be an early-season benefit to the addition of dicamba to previously researched residual herbicides + glyphosate. This is important since the critical weed-free period for soybean in Ontario is from V1 to V3 stage of growth and development (Van Acker et al. 1993).

**Density and biomass**

At 4 WAA, glyphosate/dicamba applied PP reduced GR Canada fleabane density 95% (Table 1). Glyphosate/dicamba + 2,4-D ester or paraquat reduced GR Canada fleabane density 99%; these were the only tankmixes that reduced GR Canada fleabane density more than glyphosate/dicamba applied alone. In previous research, paraquat reduced Canada fleabane...
density by 48 to 80% (Eubank et al. 2008). Saflufenacil (25 g ai ha\(^{-1}\)) + glyphosate (840 g ae ha\(^{-1}\)) has been reported to decrease Canada fleabane biomass 92%, while saflufenacil alone decreased Canada fleabane biomass 76% (Mellendorf et al. 2013). A greater decrease in Canada fleabane biomass was observed in this study, which included dicamba in the mixture.

At 4 WAA, glyphosate/dicamba applied PP reduced GR Canada fleabane biomass 99% (Table 1). Glyphosate/dicamba + 2,4-D ester reduced GR Canada fleabane biomass 99.8%, which was the only tankmix that reduced GR Canada fleabane biomass more than glyphosate/dicamba applied alone. Previous research in Nebraska observed an application of 2,4-D ester (560 g ae ha\(^{-1}\)) reduced GR Canada fleabane biomass 59 to 76% 4 WAA (Kruger et al. 2010). Research by Eubank et al. (2008) in Mississippi showed a 57 to 88% decrease in GR Canada fleabane biomass with paraquat applied PP.

Yield

Among all sites, soybean yield in the weedy control was 67% less than in the weed-free control (Table 1). Soybean yield was similar to the weed-free control in all the herbicide treatments. Eubank et al. (2008) observed a 62 to 97% decrease in soybean yield in the untreated control, when in competition with Canada fleabane, compared to the most efficacious herbicide treatment. GR Canada fleabane caused a 35 to 42% reduction in soybean yield when glyphosate (900 g ae ha\(^{-1}\)) was applied alone (Byker et al. 2013).

Future research should study the difference between herbicides with and without the addition of glyphosate/dicamba to observe if differences are significant, although there is an additional benefit to a second effective site-of-action. The addition of a second mode-of-action is recommended to delay the evolution of multiple-resistant GR Canada fleabane and other weed
species present to ensure the continued efficacy of these herbicides. Although multiple sites-of-action have been mentioned in this study, biotypes can be cross-resistant, meaning that they are resistant to more than one herbicide within the same mode-of-action (Cobb and Reade 2010). In that case, herbicides with multiple modes-of-action may be preferred to reduce selection pressure. The formulation used in this study was a premix of glyphosate/dicamba and while there are stand-alone formulations of dicamba, the addition of glyphosate to dicamba has been observed to increase control of GR Canada fleabane in research by Spaunhorst et al. (2013) and Johnson et al. (2010). However, Flessner et al. did not observe an increase in control with the addition of glyphosate to dicamba. This may be due to the percentage of glyphosate-susceptible (GS) to GR Canada fleabane within the population. However, adding glyphosate would reduce the selection pressure for dicamba-resistant broadleaf weeds.

Previous research observed unacceptable control of GR Canada fleabane with glyphosate + fomesafen (900 + 240 g ae ha\(^{-1}\)), glyphosate + acifluorfen (900 + 600 g ae ha\(^{-1}\)) or glyphosate + chlorimuron-ethyl (900 + 9 g ae ha\(^{-1}\)) applied POST. McCauley and Young (2016) observed acceptable control of GR Canada fleabane (24 cm in height) with dicamba (280 g ae ha\(^{-1}\)) applied POST, corroborating the results seen in this study. While not utilized in this study, glufosinate has been observed to control GR Canada fleabane 61 to 76% 6 WAA, the addition of dicamba improved control, providing 91 to 99% control 6 WAA (Norsworthy et al. 2009). Glyphosate + dicamba was more efficacious, controlling 97 to 99% of GR Canada fleabane 6 WAA. In this study, glyphosate/dicamba + paraquat provided ≥97% control throughout the growing season. However, due to the recent deregistration of paraquat in Canada, this option will not be commercially available. Glyphosate/dicamba with the addition of either saflufenacil, saflufenacil/dimethenamid-P or saflufenacil/imazethapyr provided ≥96% control during the
critical weed-free period and should be used for the control of GR Canada fleabane in
glyphosate/dicamba-resistant soybean. Dicamba was an acceptable tank-mix partner for the
control of GR Canada fleabane and is one option to consider for weed management in
glyphosate/dicamba-resistant soybean.

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<th>4 WAA</th>
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**Note:** Abbreviations: WAA, weeks after application.

\(^{a}\)Means followed by the same letter with a column are not significantly different according to Fisher’s Protected LSD (P ≤ 0.05).

\(^{b}\)Control estimates based on comparisons made to the weedy control.

\(^{c}\)Merge at a rate of 1 L ha\(^{-1}\) was added to the tank.