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Single and sequential applications of dicamba for the control of glyphosate-resistant common ragweed in glyphosate- and dicamba-resistant soybean

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Abstract: Confirmation of glyphosate-resistant (GR) weeds in southwestern Ontario has led to a change in weed management practices, particularly in soybean. Registered soil applied herbicides have been identified that have activity on GR common ragweed, however, due to the long emergence period of common ragweed, additional postemergence options are required. The recent development of glyphosate- and dicamba-resistant soybean (Roundup Ready Xtend soybean) allows for the preplant and postemergence application of dicamba. Three field studies were conducted in Ontario, Canada in a field with confirmed GR common ragweed. GR common ragweed interference resulted in 75% yield loss in soybean compared to the weed-free check. At 4 weeks after application, dicamba tank-mixed with glyphosate, applied preplant only, postemergence only, or preplant followed by postemergence controlled GR common ragweed up to 94, 87 and 99%, respectively. The availability of dicamba for use in glyphosate- and

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Abbreviations: DAPOST, days after post-emergence application; **GR**, glyphosate-resistant; **POST**, post-emergence; **PP**, preplant; **PRE**, pre-emergence.

dicamba-resistant soybean will provide an additional mode of action for weed management in soybean.

Key words: Glyphosate resistance, multiple herbicide-resistant crops, preplant herbicides, preemergence herbicides, postemergence herbicides

Introduction

Finding new herbicides and new herbicide modes of action is extremely rare. No new mode of action has been registered for use in agriculture in the past 20 years (Duke 2011). The development of herbicide resistant cultivars has been widely used by plant breeders and agricultural industry to address current weed management challenges. This weed management approach has diminished herbicide discovery, but broadened the context in which the herbicides could be used. The development of transgenic herbicide resistant soybean is beneficial for weed management as it increases the modes of action available for weed control. Glyphosate- and dicamba-resistant soybean is a recently available crop technology that is available to Canadian growers for weed management.

In 1996, glyphosate-resistant soybean and canola were released, followed by corn, and cotton (Dill 2005). The introduction of this technology impacted weed management strategies by providing a simple, economical, broad spectrum weed control option that could be applied from early preplant to preharvest burndown. Glyphosate use increased as the number of hectares seeded in glyphosate-resistant crops increased, resulting in intense selection pressure for glyphosate-resistant weeds. The introduction of glyphosate- and dicamba-resistant soybean came at a time when GR weeds are an increasing concern and alternative modes of action to

glyphosate are being revisited for control of these weed biotypes. Although dicamba does not control grasses, it provides broad spectrum broadleaf weed control (Green and Owen 2011), including GR biotypes. A key benefit of dicamba is that it has short-term residual activity in the soil which will reduce the need for multiple applications of dicamba per year, unlike glyphosate which has no residual activity in the soil leading to multiple applications (Green and Owen 2011). This will also be addressed in the stewardship programs being developed alongside the sale of glyphosate- and dicamba-resistant soybean.

Dicamba was introduced around 1963 for the control of annual, biennial and perennial broadleaf weeds (EU Pesticides database 2016). Dicamba is a group 4, synthetic auxin herbicide, which dicamba turns on genes that are otherwise repressed, resulting in uncontrolled plant growth and death. Dicamba and other synthetic auxin herbicides have been used for decades for broadleaf weed control in monocot crops that are generally tolerant. However, dicamba could not be used in broadleaf crops prior to the introduction of the gene that metabolizes dicamba in soybean and cotton.

Studies have identified alternative herbicides for control of GR weeds, including GR giant ragweed, Canada fleabane, and common ragweed in soybean in Ontario. However, all of these studies have concluded that there are very few effective postemergence herbicide options for effective control of these weed biotypes after crop emergence. The prolonged emergence period of these species, in particular common ragweed, poses a serious weed management problem as these weeds may not be emerged at the time of preplant herbicide application (Bassett and Crompton 1975; Stoller and Wax 1973). Previous studies have shown that use of dicamba in glyphosate- and dicamba-resistant soybean is an effective management system for giant ragweed and Canada fleabane (Vink et al. 2012; Byker et al. 2013). The objective of this study was to

evaluate pre-plant, post-emergence, and sequential applications of glyphosate and/or glyphosate plus dicamba for the control of GR common ragweed in glyphosate- and dicamba-resistant soybean.

Materials and Methods

Three field experiments were conducted over a two-year period (2014 and 2015) in a field with confirmed GR common ragweed near Windsor, Ontario, Canada. All three experiments were conducted in the same field, as this was the only location in the area with glyphosate-resistant common ragweed in sufficient quantities for conducting a trial. The two trials conducted at the site in 2015 were separated in time, rather than location. Previously, this field had a soybean-soybean-winter wheat rotation. This population is resistant to ALS (acetolactate synthase) inhibitors and EPSPS (5-enolpyruvyl shikimate-3-phosphate synthase) inhibitors herbicides (Van Wely et al. 2014a). Van Wely et al. (2014b) reported up to a 28-fold increase in the amount of glyphosate required for a 50% reduction in weed biomass for the biotype at this location compared to the susceptible biotype. The clay soil at this site contains 3.1% organic matter, with pH of 7.2. Each trial contained 3 replicates, with a weedy and non-treated weedy check in each replicate. The field was vertically tilled in the fall, and then soybeans were planted no-till in the spring following preplant applications. Soybeans were planted at 30 inch row spacing, at a population of 140,000 seeds acre⁻¹. The soybean variety was a stewarded dicamba- and glyphosate- tolerant variety provided by Monsanto Canada for research purposes only. Plots were 2.25 m by 7 m long, and were arranged in a randomized complete block design. Herbicide application timing and common ragweed size, stage, and density are presented in Table 1. Herbicides were applied using a CO₂-pressurized backpack sprayer calibrated for an application

rate of 200 L ha⁻¹ of liquid at 210 kPa. The 1 m-wide boom had three ULD 120-20 flat fan nozzles (Hypro, New Brighton, MN) spaced 50 cm apart.

Treatments were applied either prior to soybean planting (PP) or following crop emergence (POST) or at both timings (SEQUENTIAL). Two rates of dicamba (Banvel II®, 480 g a.e L⁻¹, diglycolamine salt of dicamba, BASF Canada Inc., Mississauga, ON), 300 and 600 g a.e. ha⁻¹, were tank-mixed with glyphosate (Weathermax®, Monsanto Canada Inc., Winnipeg, MB) at 900 g a.e. ha⁻¹ (Table 2). Soybean injury and weed control ratings were taken at the POST application timing, and 10 and 28 days after POST (DAPOST), and at soybean maturity. Control ratings were visually estimated using a 0 (no control) to 100 (complete control) scale. Glyphosate-resistant common ragweed density and dry weight data was collected at 28 days after the POST application by counting and cutting the plants at the soil surface from two 0.25 m² quadrats per plot, plants were weighed following drying in a kiln. Soybean was harvested at maturity and yields were standardized at 13% moisture and presented as a percentage of the weed-free check (Table 2).

Data were analyzed using the PROC MIXED procedure for ANOVA analysis in SAS. Variances were divided into fixed and random effects. Herbicide treatment was a fixed effect, while random effects included environment (location and year), replication within environment, and the treatment by environment interaction. The significance of the environment, replication, and interaction of the environment by treatment was tested using the Z test. The significance of the fixed effects was tested using the F test. Natural log, square root, and arcsine square root transformations of the data were used where necessary for data analysis, and then back transformed for presentation of the results. Data was combined across all site years since the

interaction between environment and treatment was non-significant. Treatment means were separated using Fisher's Protected LSD at $p = 0.05$.

Results and Discussion

At 28 DAPOST, glyphosate applied as a single application (PP or POST) or in sequence, controlled GR common ragweed 30 to 64% compared to non-treated weedy control. Similarly, GR common ragweed dry weight and density decreased 45 to 65 % and 37 to 54 %, respectively, compared to the non-treated weedy control (Table 2). Since glyphosate did not provide 100% control, it confirms that a portion of this population is resistant to glyphosate. This provides the basis to demonstrate the added benefit of dicamba in the tank-mix, compared to only using glyphosate. Glyphosate selectively eliminates the susceptible plants in all treatments.

At 28 DAPOST, glyphosate ($900 \text{ g a.e. ha}^{-1}$) plus dicamba applied at 300 or $600 \text{ g a.e. ha}^{-1}$ controlled GR common ragweed 89 and 94%, respectively. Dicamba tank-mixed with glyphosate applied POST at 300 and $600 \text{ g a.e. ha}^{-1}$ controlled GR common ragweed 70 and 87%, respectively. A PP or POST application of dicamba reduced GR common ragweed dry weight $\geq 72\%$ and density $\geq 82\%$. This is equivalent to control from applications of linuron and metribuzin applied PP and fomesafen applied POST (Van Wely et al. 2014c), which are the other currently known options for GR common ragweed control.

Control of GR common ragweed was most consistent with sequential applications of dicamba. At 28 DAPOST, dicamba tank-mixed with glyphosate PP followed by (fb) POST provided greater than 98% control. Sequential applications of dicamba tank-mixed with glyphosate reduced GR common ragweed dry weight and density by $\geq 99\%$ compared to the non-treated weedy control (Table 2). Soybean yield ($\geq 96\%$ of the weed-free check) reflected the

improved GR common ragweed control with sequential applications of dicamba. Overall, there was no significant difference in soybean yield among all treatments that included at least one application of dicamba; however, soybean yield was lower when only a single application of glyphosate was used (Table 2). GR common ragweed interference reduced soybean yield by 75% in the weedy control, showing the highly competitive nature of common ragweed in soybean, and a need for season long control.

Dicamba tank-mixed with glyphosate PP provided more consistent GR common ragweed control which resulted in higher soybean yield. In addition, the application of dicamba at this time of the year will result in less off-site injury, as sensitive crops in adjacent fields are less likely to have emerged. Although, glyphosate- and dicamba-resistant soybean does not introduce a new herbicide, it does provide a new herbicide option in soybean and increases the tools available for resistance management; both for delaying, as well as controlling GR biotypes. Barnett et al. (2013) found that the addition of dicamba or 2,4-D to glufosinate or fomesafen (86-93% control) resulted in greater control of giant ragweed at 30 days after herbicide application (DAA), (56-61% control). Dicamba is another option for control of GR common ragweed in glyphosate- and dicamba-resistant soybean.

Prevention and management of GR weeds requires a long-term diversified weed management program. Previous studies reported excellent control of GR common ragweed with PRE herbicides that have long residual such as linuron and metribuzin, or sequential applications of herbicides such as PP herbicide followed by fomesafen applied POST (Van Wely et al. 2014b). However, in each of these cases, fomesafen was the only herbicide that provided effective post-emergence control of GR common ragweed. In addition, the rate of metribuzin required is costly and may result in soybean injury. Thus the use of dicamba in glyphosate- and

dicamba-resistant soybean for control of GR common ragweed provides an additional mode of action applied PP and/or POST.

With the current GR weeds in Ontario, Canada, there are few POST herbicide options for their control in soybean. Studies have shown that overall weed densities decreased when at least one herbicide, such as dicamba, was added to glyphosate (Akers et al. 2014). Glyphosate- and dicamba-resistant soybean will allow for the use of an alternative herbicide in soybean production. Weed management studies continue to show that the greater the number of effective modes of action used on a species within a field, the less chance there is that the resistant biotypes will be selected for within a population.

Previous studies on GR giant ragweed and Canada fleabane concluded that there are no effective POST herbicide options in soybean (Vink et al. 2012, Byker et al. 2013). In the past, cloransulam was considered as an alternative herbicide to glyphosate, however evolution of ALS inhibitor resistance has also been reported in these GR populations. Therefore, the recent introduction of glyphosate- and dicamba-resistant soybean will facilitate more sustainable herbicide site of action rotations and mixtures, decrease the reliance on glyphosate, and provide an effective POST option in soybean (Heap 2014). Green and Owen (2011) indicate that in addition to the use of herbicide resistant crops, new herbicide modes of action need to be discovered along with other weed management tactics to develop long-term sustainable weed management programs.

Integrated weed management is key to manage and prevent the selection of herbicide resistant weeds. Heap et al. (2014) defined integrated weed management as the inclusion of all economically available weed control measures, of which herbicide resistant crops is one of many. When looking at the ecology of herbicide resistance, the weed management techniques are

what are often focused on. However, Duke and Powles (2009) support Heap's phrase "all economically available weed control techniques", highlighting that growers will adopt technologies that provide them with a cost savings, better weed management, and simplicity of use. Until confronted with resistance, growers know that herbicides work with minimal effort and that glyphosate has great flexibility. However, we cannot use this as an excuse to rely solely on herbicides for weed control.

Herbicide resistant crops are a tool, but cannot be the only solution for controlling GR and other herbicide resistant weed species. Herbicide resistant crops can be a key as they allow for POST herbicide application in crop, without risk of crop injury, and provide an alternative mode of action for control of weeds that emerge alongside or following crop emergence. Although there are 26 cases of synthetic auxin resistant weed biotypes worldwide, the infested acreage is low (Heap 2016). However, despite the limited spread of resistance to these synthetic auxins after decades of use, it would be unwise to say that it will not happen, as was thought when GR crops were released following two decades of non-crop use. Weeds will adapt to the selection pressure and evolve resistance to any herbicide if it is continuously used as the key method of weed control (Green and Owen 2011).

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Table 1. Herbicide application timing and common ragweed stage, size, and density at time of herbicide applications for Windsor site with glyphosate-resistant common ragweed in 2014 and 2015.

Location	Year	Application date		Common ragweed		Application date		Common ragweed	
		Preplant	Size (cm)	Leaf Number	Density (m ⁻²)	Postemergence	Size (cm)	Leaf Number	Density (m ⁻²)
S1	2014	22-May-14	up to 5 cm	up to 6 lf	253	26-Jun-14	up to 15 cm	up to 12 lf	182
S2	2015	16-May-15	up to 5 cm	up to 4 leaf	548	28-May-15	up to 10 cm	up to 8 leaf	488
S3	2015	21-May-15	up to 8 cm	up to 6 leaf	402	3-Jun-15	up to 12 cm	up to 10 lf	398

Table 2. Control of glyphosate-resistant (GR) common ragweed with glyphosate and dicamba tank-mixed with glyphosate in PRE, POST, or SEQUENTIAL applications at 10 and 28 days following treatment application (DAPOST). Dry weights and density are presented as percent of the non-treated weedy check. Yield data is expressed as a percentage of the weed-free check and data is back transformed from a square root transformation.

Treatment	Rate (g ae ha ⁻¹)	Timing	GR Common ragweed control (%)		Dry wt. (%)	Density (%)	Yield (%)
			10 DAPOST	28 DAPOST			
Non-treated weedy check							25 b
Weed-free check							100 ab
Preplant Application							
Glyphosate	900	PP	30 f	30 e	55 d	63 d	43 b
Glyphosate + dicamba	900+ 300	PP	91 abc	89 ab	1 a	2 a	94 ab
Glyphosate + dicamba	900+ 600	PP	96 abc	94 ab	0 a	1 a	84 ab
POST Application							
Glyphosate	900	POST	32 ef	48 d	49 cd	46 c	52 b
Glyphosate + dicamba	900+ 300	POST	47 de	70 c	28 b	18 b	59 ab
Glyphosate + dicamba	900+ 600	POST	57 d	87 b	3 a	4 a	66 ab
Sequential Application							
Glyphosate fb glyphosate	900, 900	PP POST	60 d	64 c	35 bc	52 cd	69 ab
Glyphosate fb glyphosate + dicamba	900, 900 + 300	PP POST	77 c	89 ab	4 a	5 ab	84 ab
Glyphosate + dicamba fb glyphosate	900 + 300, 900	PP POST	90 abc	97 ab	0.2 a	0.8 a	94 ab
Glyphosate fb glyphosate + dicamba	900, 900+ 600	PP POST	81 bc	96 ab	0.5 a	0.1 a	89 ab
Glyphosate + dicamba fb glyphosate	900+ 600, 900	PP POST	99 a	93 ab	0.1 a	1.2 a	92 ab
Glyphosate + dicamba fb glyphosate + dicamba	900+ 300, 900+300	PP POST	98 a	98 ab	0 a	0 a	96 ab
Glyphosate + dicamba fb glyphosate + dicamba	900+300, 900+600	PP POST	97 ab	99 ab	0 a	0.3 a	104 a
Glyphosate + dicamba fb glyphosate + dicamba	900+600, 900+300	PP POST	99 a	97 ab	0 a	0.6 a	97 ab

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