Final Report

Quantifying the risks associated with late and sequential herbicide applications in herbicide-resistant canola systems

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Abstract

Herbicide-resistant (HR) canola has been widely adopted and now dominates the canola market in Canada. Recent concern regarding the tolerance of HR canola crops to late herbicide applications has piqued producer interest, as well as that of agronomists, in the timing of herbicide applications. Although late applications of glyphosate have been shown to have detrimental effects on cotton, soybean, and corn, little is known about the effects of late herbicide applications on canola reproduction, seed set, yield, and quality. The objectives of this research were to determine: 1) the response of HR canola to late and sequential herbicide applications with regard to reproductive ecology, yield, and yield components; 2) differences in crop tolerance among the different HR canola systems in several environments; 3) to identify the cause(s) of any reductions in crop tolerance. Experiments conducted over 8 site-years across Alberta and Saskatchewan showed the response of HR canola systems to late herbicide applications was ambiguous. The glyphosate-resistant system appeared to be impacted more significantly than either the glufosinate-resistant or imidazolinone systems. There is little flexibility in the glyphosate-resistant system for late, off-label applications and these must be avoided whenever possible. If producers are forced to make late applications due to inclement weather and in these circumstances, producers must weigh the perceived yield loss due to emerged weeds against the potential for sizeable reductions in yield before applying herbicides late in canola crops.

Background

The ability of herbicide-resistant (HR) canola to control a broad spectrum of weeds has led to rapid adoption of the technology, with over 90% of cultivars grown in western Canada being HR (O'Donovan et al., 2006). The suggested window for the application of herbicides in canola ranges from cotyledon to early bolting stages, but each herbicide within each HR system has its own specific requirements, and this may be confusing producers, resulting in herbicides being applied too late. For example, in-crop applications of glyphosate can be made until the sixth-leaf stage in glyphosate-resistant (GLYR) canola varieties whereas glufosinate can be applied until the early bolting stage in glufosinate-resistant (GLUR) canola varieties (Brook and Cutts, 2009). Ideally, herbicides should be applied as early as possible to prevent yield loss from early emerging weeds. In practice, however, herbicides applied to early may miss later-emerging weeds, which may then warrant further (sequential) herbicide applications (Schilling et al., 2006). Additional constraints such as adverse environmental conditions can also force producers to apply herbicides at suboptimal growth stages, which could compromise crop tolerance and result in reductions in crop yield and quality.

In theory, HR canola varieties should tolerate with minimal injury the application of a herbicide to which they have engineered resistance. Several recent reports have indicated, however, that there may be problems associated with late and sequential herbicide applications in HR canola crops (Anonymous 2006; Barker, 2007). Only anecdotal evidence suggests that crop safety may be compromised with late and sequential herbicide applications; there is no scientific literature that examines the response of HR canola to late and sequential herbicide applications with regard to the reproductive biology of the crop, crop yield and quality. Given that delayed herbicide applications to other HR crops (soybean, cotton, corn) causes reduced growth (Young et al., 2001; Pline et al., 2003; Norsworthy, 2004), altered reproductive morphology, male sterility, and reductions in seed set (Pline et al., 2002a,b; Thomas et al., 2004), similar issues may exist in HR canola.

As producers strive to grow more canola more often to meet the rising demands of the crushing and biofuels industries, the likelihood of herbicides being applied late in the life cycle of the canola crop will increase. The recommendations developed from this research should help to address this issue by bringing to light any crop tolerance issues (and causes) associated with applying herbicides too close to the reproductive phase. In this regard, the results of the proposed research will be integral to maximizing canola yield and quality while minimizing crop injury, thus providing the highest possible economic returns to growers and the highest quality canola seed to the crushing industry. This project will, for the first time, provide empirical data on the issue of late herbicide applications in canola and therefore, the recommendations emerging from this research should deter producers from applying herbicides when canola crops are nearing the reproductive stage.

This research builds on research conducted in Alberta by Clayton et al. (2002) and Schilling et al. (2006) who reported reduced seed and biomass yields, respectively, when glyphosate was applied to glyphosate-resistant canola at the six-leaf compared with the two-leaf stage. However, previous studies were designed to examine time of weed removal effects and could not assess whether yield loss under field conditions was due to a longer duration of weed competition or inadequate crop tolerance to a late herbicide application. The project focued on isolating HR canola crop sensitivity to herbicides in all HR canola systems to determine if crop tolerance is compromised with late or sequential herbicide applications.

Therefore, the following objectives were addressed:

1. What is the response of HR canola to late and sequential herbicide applications with regard to reproductive ecology, yield, and yield components?
2. Are there differences in crop tolerance among the different HR canola systems in several environments to develop recommendations specific to each HR canola system?
3. What are the cause(s) of any reductions in crop tolerance and are there practical solutions?

Materials and Methods

The research was conducted at two locations across Alberta (St. Albert, Lacombe) from 2010-2012 and at one location in Saskatchewan (Saskatoon) in 2012. Although Lethbridge originally indicated that they had the capacity to run a site in 2010, logistical issues prevented this. The experimental design at all sites was a randomized complete block with four replications per treatment (plot size 2 x 6 m) conducted on either barley stubble (Edmonton) or fallow (Lacombe, Saskatoon). In 2010, an early spring frost forced the trial to be reseeded at Lacombe towards the end of May while planting occurred in early May at the Edmonton site.

Prior to plot establishment, trifluralin was applied as a pre-plant incorporated treatment in the spring to provide residual weed control. Rates were determined based on soil type, soil texture and organic matter but were generally around 1435 g a.i. ha^{-1}. The entire plot area was then tilled twice in opposite directions with a field cultivator to a depth of 10 to 12 cm to incorporate the trifluralin. Glyphosate-resistant (GLYR), glufosinate-resistant (GLUR), and imidazolinone-resistant (IMIR) canola cultivars were planted at a rate of 150 seeds m^{-2}, with fertilizer side-banded at rates recommended based on soil test results. Glyphosate, glufosinate, and imazamox herbicides were applied as a single application at the two-leaf (2L), six-leaf (6L), bud (B), and the early-bloom (EB) stages of canola (Table 1); sequential applications were applied at the 2L&6L, 2L&B, 2L&EB; an unsprayed treatment was included as a check. Rates of glyphosate, glufosinate, and imazamox application were 450, 500, 20 g a.i. ha^{-1} in single applications, respectively, and 900, 1000, 40 g a.i. ha^{-1} (total) in sequential applications. Plots were hand-weeded weekly to maintain weed-free plots. Canola varieties used in the study were 45H28 (RR), 45H73 (CF), and Invigor 5440 (LL).

Crop densities were determined by counting plants in two 1 m rows, 3-4 weeks after crop emergence. Yield component data was collected, including the number of fully formed and aborted pods per plant, as well as the number of seeds per pod. At physiological maturity but prior to harvest, five random plants in each plot were cut at the soil surface and carefully removed from each plot. Plant height was recorded on each of these plants, and the number of fully formed and aborted pods was also enumerated. Following this, five pods were randomly chosen for removal from each of the five plants, and the number of seeds in each of these five pods was recorded. Canola seed yield was harvested with a small plot combine from the central six rows of each 8-row plot. Samples were then dried to a constant moisture, cleaned, and the weight of the grain recorded. From this sample, the weight of 250 seeds was determined and multiplied by a factor of four to provide an estimate of thousand seed weight (TSW).

With regard to the statistical analysis, residuals were initially tested to ensure that they conformed to the assumptions of analysis of variance, namely homogeneity of error variance and normality. With the assumptions met, the data were subjected to ANOVA to test for main effects and interactions using the GLM procedure of Statistical Analysis Software (SAS) (SAS Institute, 2011). Because of the large differences and between site-years, all data were analyzed within site-years. Means were separated by a Dunnett's test, with treatment effects declared significant at $P \leq 0.05$. Dunnett's test is used to simultaneously compare all treatments to a single control treatment.

Table 1. Herbicide-resistant system and application timing of various treatments.

Treatments	Herbicide-resistant System	Herbicide application timing
1.	Glyphosate (Roundup Ready)	2L
2.	Glyphosate	6L
3.	Glyphosate	B
4.	Glyphosate	EB
5.	Glyphosate	2L&6L
6.	Glyphosate	2L&B
7.	Glyphosate	2L&EB
8.	Glufosinate (Liberty Link)	2L
9.	Glufosinate	6L
10.	Glufosinate	B
11.	Glufosinate	EB
12.	Glufosinate	2L&6L
13.	Glufosinate	2L&B
14.	Glufosinate	2L&EB
15.	Imidazolinone (Clearfield)	2L
16.	Imidazolinone	6L
17.	Imidazolinone	B
18.	Imidazolinone	EB
19.	Imidazolinone	2L&6L
20.	Imidazolinone	2L&B
21.	Imidazolinone	2L&EB

Results

All sites - 2012

Statistically significant differences in yield were not seen at the Saskatoon, Lethbridge, or Lacombe locations at any stage of application of either glyphosate or imazamox (Figs 1-2). Application of glufosinate at the 2-leaf and 6-leaf stage sequentially, resulted in a statistically significant yield loss at Saskatoon, and an economic reduction of $193/acre. Although not statistically significant, yield differences in Saskatoon were economically significant. Similar data was seen in earlier years at Edmonton and Lethbridge (2010-2011), where economically significant, but not statistically significant differences were observed between treatments (Figs 3-6). In 2011, applications of glufosinate to GLUR canola at the 2-leaf and early blooming stages combined reduced economic yield by up to $180/ac at Lethbridge (Fig 3). Similarly, in 2011, applications of glufosinate to GLUR canola at the 2-leaf and early blooming stages combined reduced economic yield by up to $113/ac at Edmonton. Similar results were found at Lacombe and Edmonton in 2010 (Fig 4).

Applications of glyphosate at Saskatoon in 2012, at the 2-leaf and early bloom stages, sequentially, resulted in the largest economic yield loss at $91/ac when compared to all other stages (Fig 2). Contrary to the results in previous years (Figs 5-6), applications of glyphosate in 2012, after the 6-leaf stage, did not result in a significant decrease in economic yield.

Significant differences in yield were not seen within individual growth stages at the Lethbridge (Fig. 2) and Lacombe (Fig. 3) locations, but were present when comparing groups of herbicide timing applications with the control or with each other (Table 2). At the Lethbridge location, significant differences were seen within “On label vs Control” (+1466 kg/ha) and “Single vs Control” (+1416 kg/ha) in the glufosinate treatments, and within “On label vs Control” (-552 kg/ha), “Double vs Control” (-537 kg/ha), “Double early vs Double late” (-420 kg/ha), and “Double early vs Control” (-817 kg/ha) in the glyphosate treatments (Table 3). At the Lacombe location, significant differences in yield were only present within the “Double vs Control” (+452 kg/ha) and “Double late vs Control” (+448 kg/ha) in the glyphosate treatments. All other stages and herbicide treatments did not produce a statistically significant difference. No significant contrasts were found at the Saskatoon site.

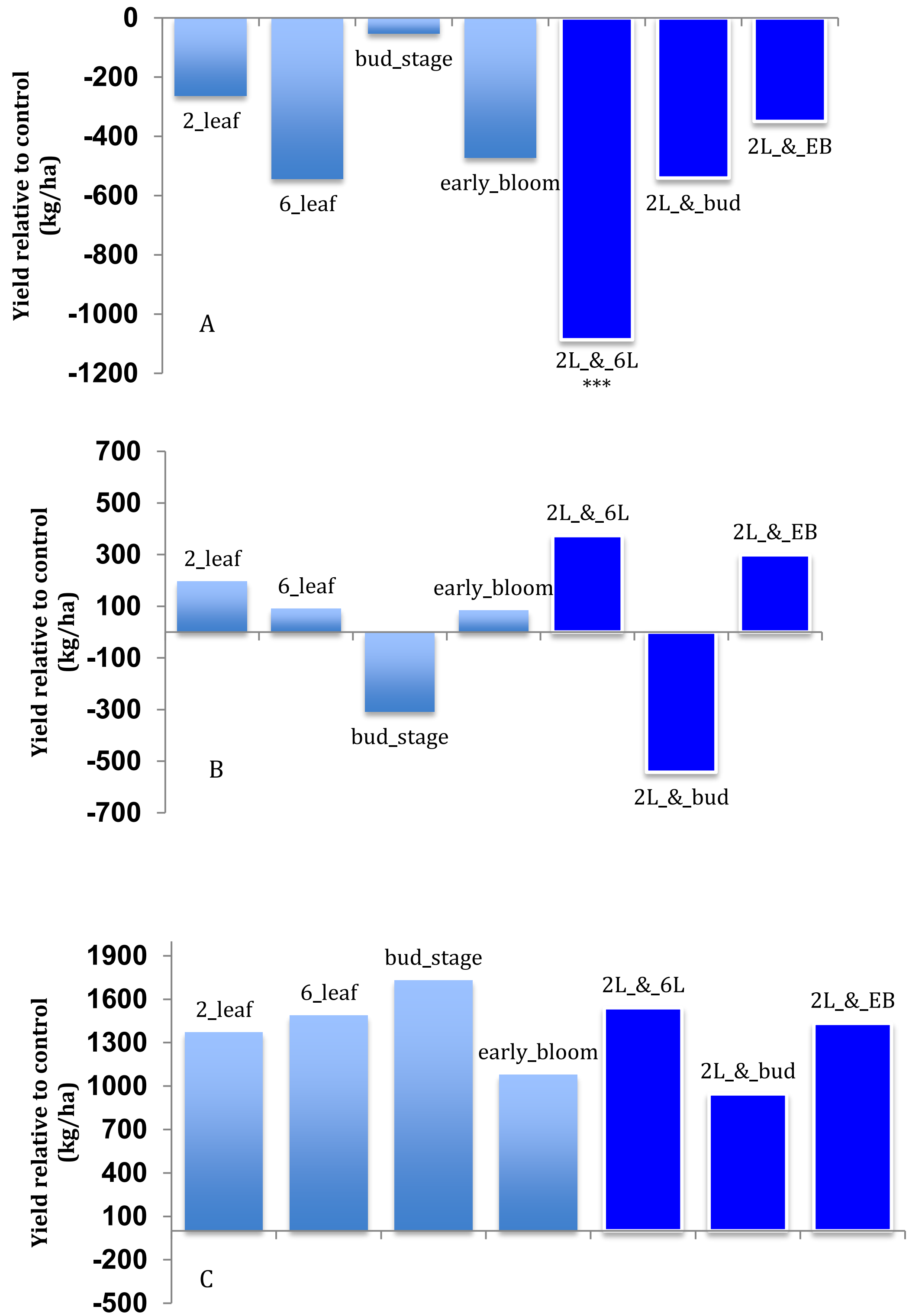

Figure 1. Response of glufosinate-resistant (Liberty Link), canola yield to late and sequential applications at Saskatoon (A), Lacombe (B), and Lethbridge (C) in 2012; unsprayed check yields were 3234 kg ha^{-1}, 4296 kg ha^{-1}, 4957 kg, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2_leaf, two leaf; 6-leaf, six-leaf; bud, bolt; EB, early bloom.

Table 2. Single degree of freedom contrasts and the associated estimates of the difference between means for the GLUR system at all sites in 2012.

Comparison	Lethbridge	Saskatoon	Lacombe
On Label vs Control	1466.4*	-631.2	219.77
Off Label vs Control	1297.2	-354	-117.3
Single vs Control	1416.8*	-333.1	15.069
Double vs Control	1306.9	-659	43.342
Single vs Double	109.82	325.88	-28.27
Single-early vs Single-late	24.575	-140.5	255.49
Double-early vs Double-late	351.24	-641.6	495.54
Single-early vs Control	1429.1	-403.4	142.81
Single-late vs Control	1404.5	-262.9	-112.7
Double-early vs Control	1541.1	-1087*	373.7
Double-late vs control	1189.9	-445.1	-121.8

*Denotes statistically significant differences between comparisons at $P < 0.05$.

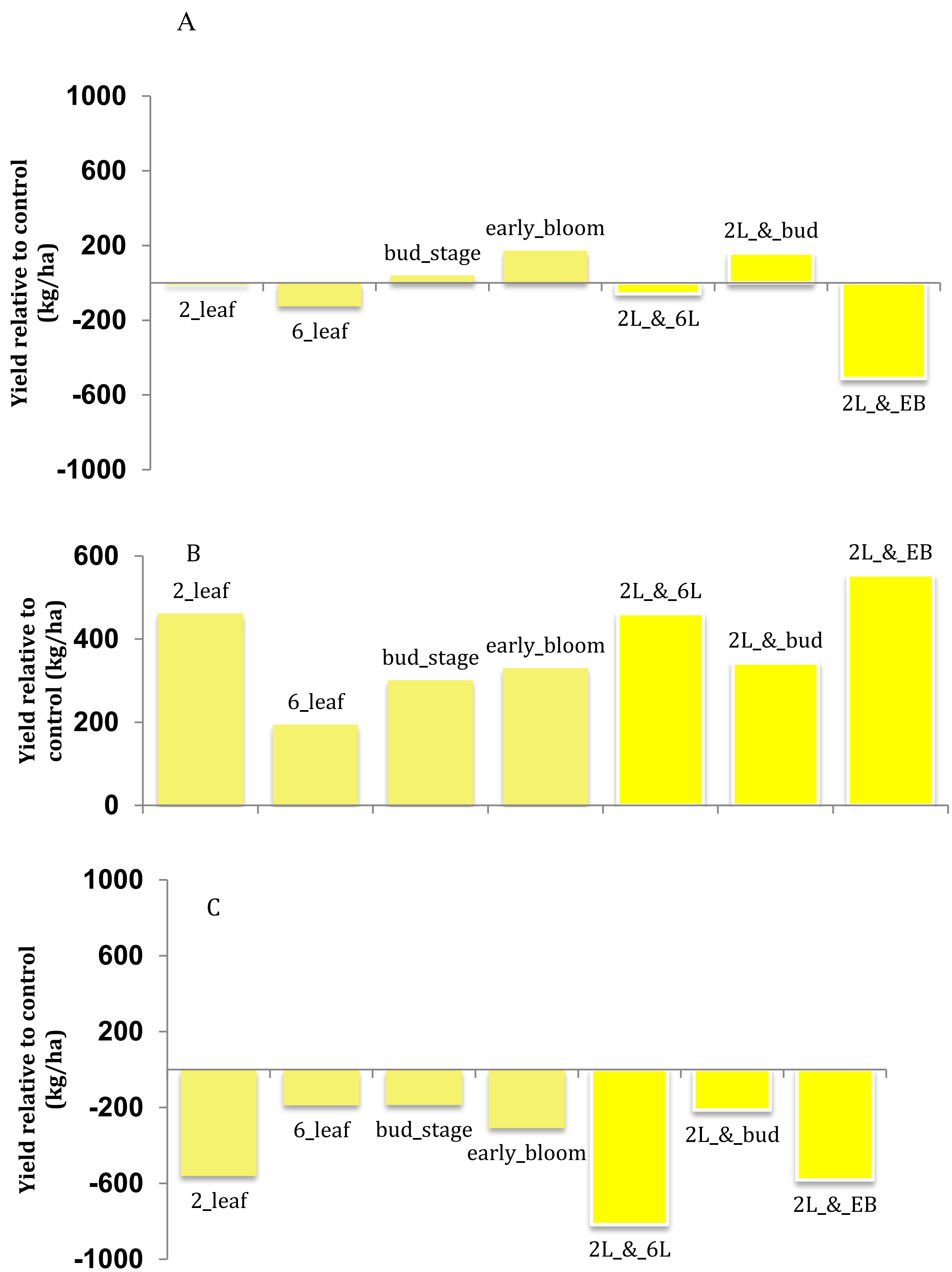

Figure 2. Response of glyphosate-resistant (RoundUp) canola yield to late and sequential applications at Saskatoon (A), Lacombe (B) and Lethbridge (C) in 2012; unsprayed check yields were 2341 kg ha^{-1}, 2638 kg ha^{-1}, and 3144 kg ha^{-1}, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2_leaf, two leaf; 6-leaf, six-leaf; bud, bolt; EB, early bloom.

Table 3. Single degree of freedom contrasts and the associated estimates of the difference between means for the GLYR system at all sites in 2012.

	Lethbridge	Saskatoon	Lacombe
On Label vs Control	-522*	-65.08	372.53
Off Label vs Control	-322.1	-35	382.16
Single vs Control	-310.7	18.875	321.98
Double vs Control	-537.2*	-136.9	452*
Single vs Double	226.53	155.79	-130.8
Single-early vs Single-late	-127.5	-174.8	12.237
Double-early vs Double-late	-420.1*	118	12.937
Single-early vs Control	-374.4	-68.5	328.1
Single-late vs Control	-247	106.25	315.86
Double-early vs Control	-817.3*	-58.25	461.4
Double-late vs control	-397.2	-176.3	448.46

*Denotes statistically significant differences between comparisons at $P < 0.05$.

Pods

No significant differences in the number of seeds/pod and number of aborted pods were seen at the Lethbridge (Fig. 3) site in all herbicide treatments except for glyphosate at the 2-leaf and early bloom stage. In the GLUR system at the 2-leaf and 6-leaf stages, a significant decrease in the number of seeds/pod (2.25) was also observed (data not shown). At the Lethbridge site, the GLUR system exhibited a decrease in aborted pods, where as the GLYR system resulted in an increase in aborted pods across all growth stages (Fig. 4). The number of seeds/pod was significantly different in “Double early vs Double late” (+2.62 seeds/pod) applications in Clearfield and “Single vs Double” (-1.5 seeds/pod) and “Single early vs Single late” (+1.62 seeds/pod) in GLYR system (data not shown). These findings mirror previous years' with a marked increase in aborted pods when applications of glyphosate are applied late or as sequential applications.

No significant differences in the number of seeds/pod and number of aborted pods were seen at the Lacombe site (Fig. 3) in all herbicide treatments except for the GLYR system (data not shown). Glyphosate applications also resulted in a decrease in the number of seeds/pod at all individual herbicide application timings. The decrease in seeds/pod and increase in aborted pods/plant at all stages, though not statistically significant, highlight the potential issues with the late herbicide applications in the 2012 growing season.

TSW

At Saskatoon, glufosinate applied at the 2-leaf and early bloom stages, sequentially, resulted in a significant decrease of 0.91g per 1000 seeds, with no other stages being significantly affected by glufosinate applications (Fig. 6). Treatments at the Lethbridge and Lethbridge location showed no significant effects of individual herbicide timing on 1000-seed weight within the GLUR system or across any of the other HR systems (Fig. 6).

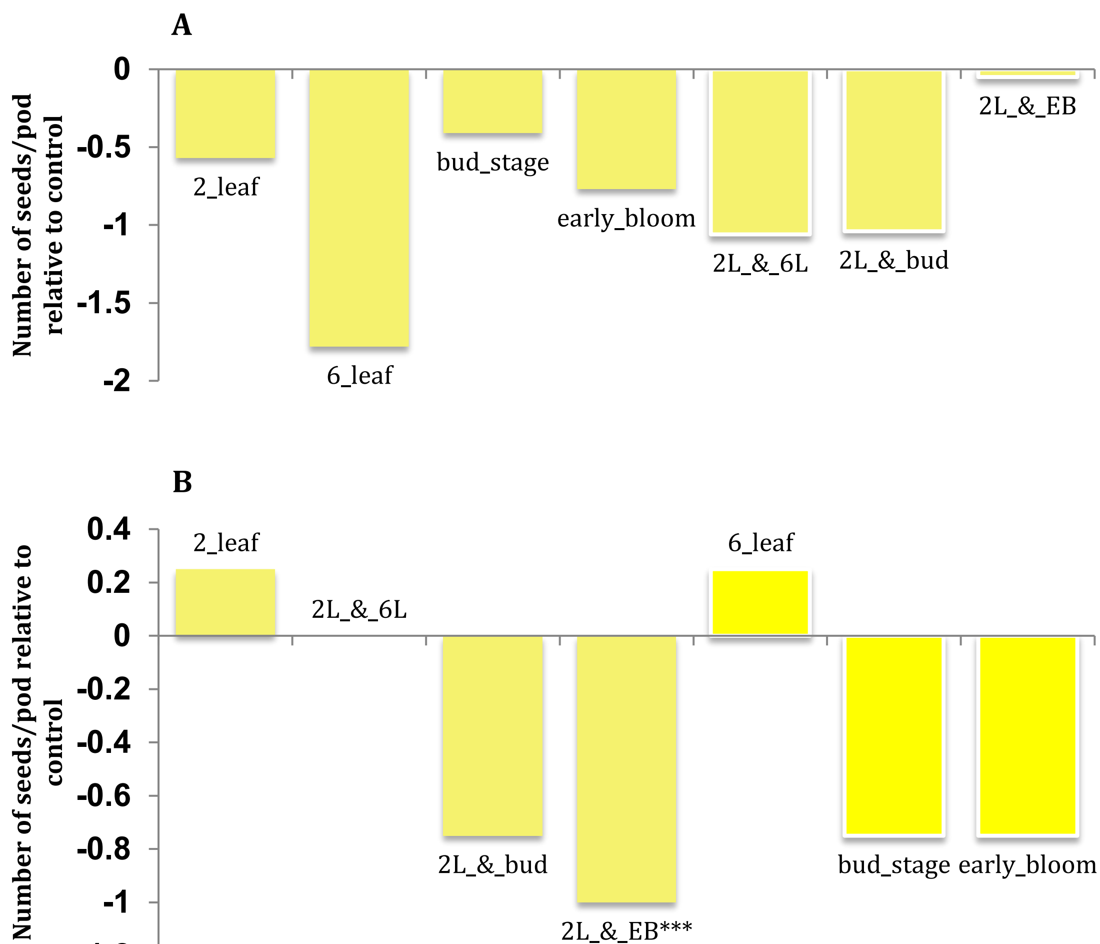

Figure 3. Seed production in glyphosate-resistant (RR) canola due to late and sequential applications of glyphosate at Lacombe (A) and Lethbridge (B), AB in 2012. Seed production in the unsprayed check averaged 27.5 seeds pod^{-1} and 29.25 seeds pod^{-1}, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2_leaf, two leaf; 6-leaf, six-leaf; bud, bolt; EB, early bloom.

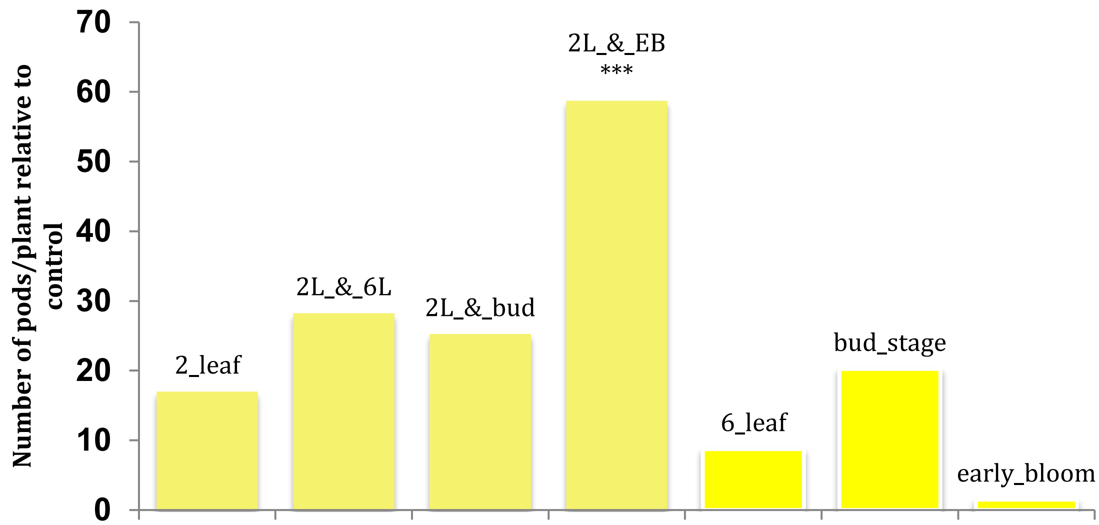

Figure 4. Pod abortion per plant in glyphosate-resistant (RR) canola due to late and sequential applications of glyphosate at Lethbridge, AB in 2012. Seed production in the unsprayed check averaged 81.5 pods/plant. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2_leaf, two leaf; 6-leaf, six-leaf; bud, bolt; EB, early bloom.

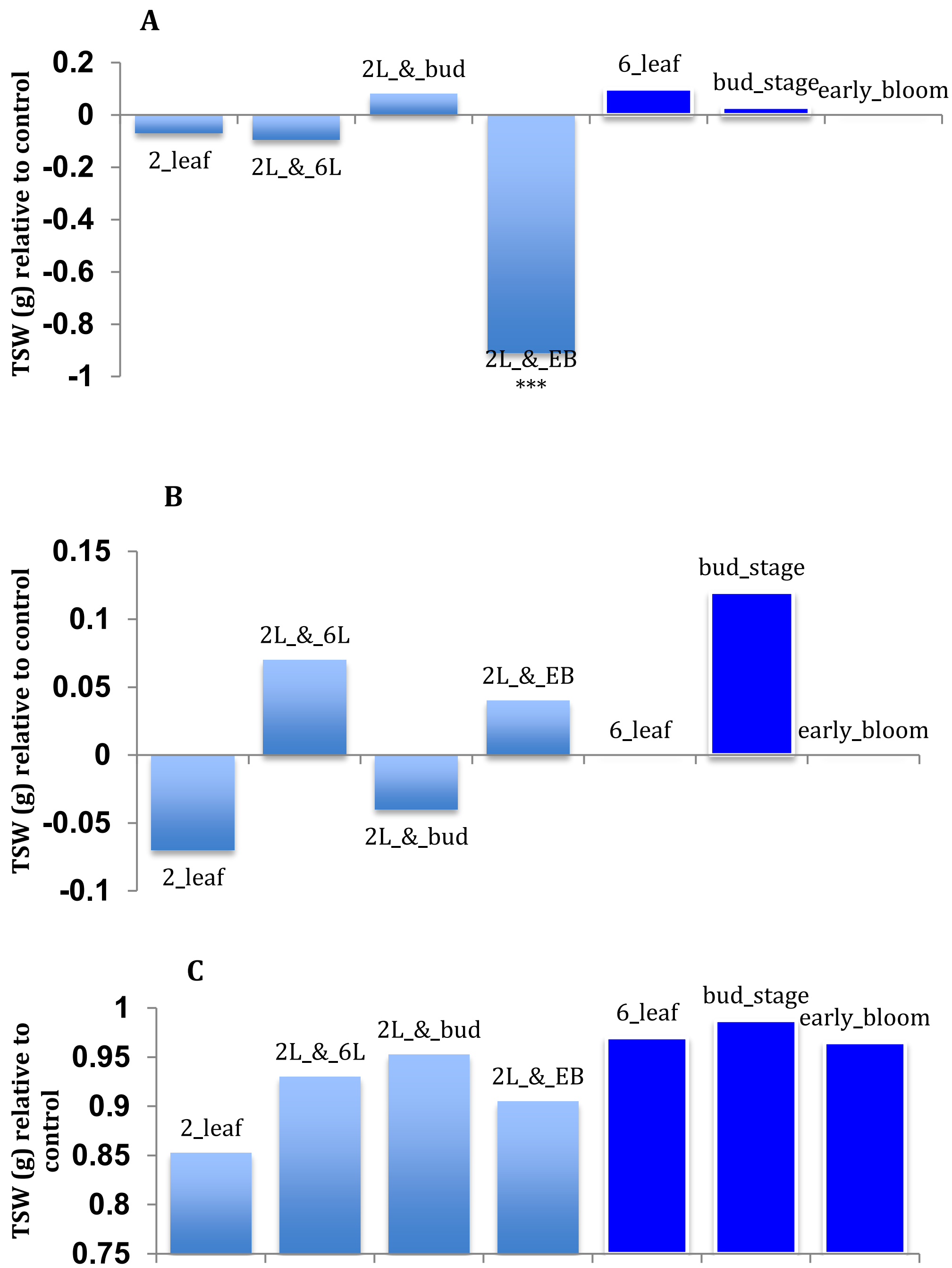

Figure 6. Thousand seed weight (TSW) in glufonsinate-resistant (Liberty Link) canola due to late and sequential applications of glyphosate at Saskatoon (A), Lacombe (B) and Lethbridge (C) in 2012. TSW in the unsprayed check averaged were 4.05g, 3.90g, and 2.83g, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

All sites - 2011

Although differences between treatments for many variables were significant at Lethbridge and Edmonton, very few differences between treatments were significant at Lacombe. This may be attributable to the cool, wet conditions at Lacombe, which favor crop growth and are sufficient for herbicide metabolism.

With respect to the GLUR system, there were generally no significant differences in most variables measured across all site-years. The one exception was plant height, where delayed applications at the bolt and early bloom stages resulted in significantly shorter plants at both Lacombe and Lethbridge (data not shown). Despite yield reductions not being statistically significant, they were economically relevant and quite substantial in some cases (Fig. 7). At both Edmonton and Lethbridge, applying glufosinate to GLUR canola at the six-leaf stage and beyond reduced yield relative to the unsprayed check plots. Both sites showed substantial yield declines when applications were made beyond the bolting stage of crop development, which represented the end of the registered window for application. Although not significant, these values are very important because the yield reduction observed when applications are made at the 2L&EB stages of canola crop development varied between 9-18 bu/ac and about \$222 to \$445 ha^{-1} would be lost at an assumed canola price of \$10/bushel. Late application trends were similar at Edmonton, although yield reductions at the Edmonton site were far greater than those at the Lethbridge site and may be due to differences in environmental conditions at the time of herbicide application. The efficacy of glufosinate is known to be affected by environmental conditions. Similar to data from the Edmonton site in 2010, yield reductions were observed when glufosinate was applied at the bolting stage, particularly when multiple applications were made. It is important to point out that an application at the early bloom stage is not registered, but both a single and sequential application at the bolt stage is registered. The sequential application may need to be reconsidered as losses were consistent and sizeable across four out of five site-years. No reductions in seed quality were observed across the three sites in 2011.

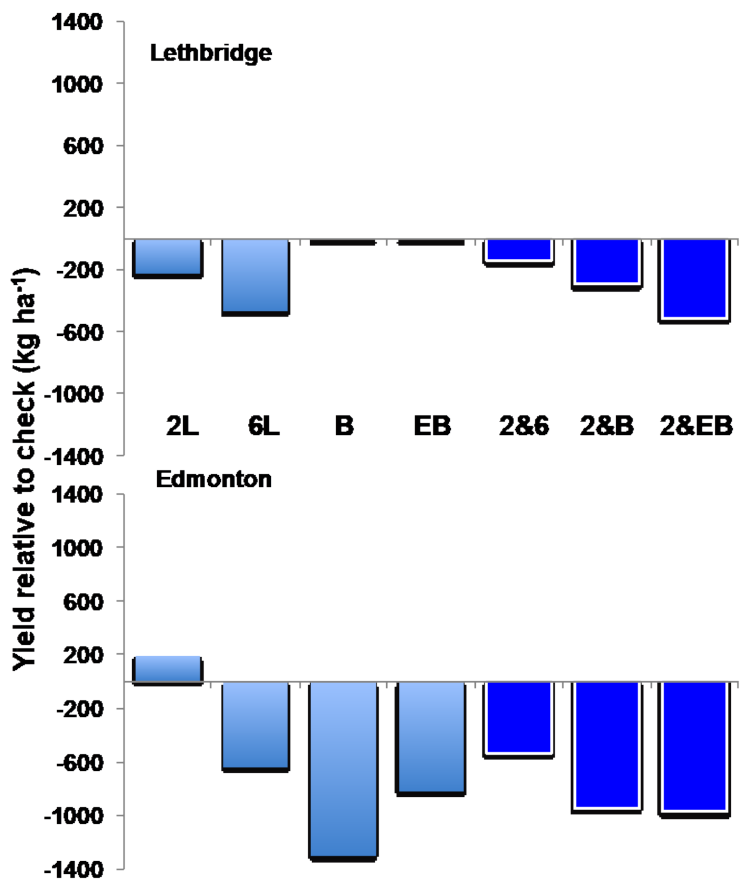

Figure 7. Response of glufosinate-resistant (LL) canola yield to late and sequential applications of glufosinate at Lethbridge and Edmonton, AB in 2011. Unsprayed check yields were 3652 kg ha^{-1} and 6114 kg ha^{-1} at Lethbridge and Edmonton, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

With regard to the glyphosate-resistant system, yield losses were observed when glyphosate was applied at the six-leaf stage and beyond (Fig. 8). At Lethbridge, a significant reduction in yield relative to the unsprayed check was observed when a single application was made at the early-bloom stage of crop growth. The Edmonton site did not see a statistically significant reduction in yield relative to the check, but single and sequential applications made at the bolt stage and beyond resulted in a significant reduction in yield relative to an application made at the two-leaf stage. Reductions in yield at the bolt stage varied from 552 kg ha^{-1} at Lethbridge to 1143 kg ha^{-1} (20 bu/ac) at Edmonton when an application occurred at the bolt stage, with similar reductions occurring at the 2L&B. Reductions in yield at the early bloom stage varied from 830 kg ha^{-1} (15 bu/ac) at Lethbridge to nearly 550 kg ha^{-1} (10 bu/ac) at Edmonton when an application occurred at the 2L&EB. Reductions in revenue from these yield declines range from \$457 ha^{-1} with an application made at the bolt stage in Edmonton to \$237 ha^{-1} when an application was made at the 2L&EB in Edmonton. Thus, a substantial amount of income would be lost if a glyphosate application were made past the bolt stage of glyphosate-resistant canola. These results are consistent with the 2010 data and suggest that late applications of glyphosate to GLYR canola results in substantial declines in crop yield. No differences in yield were observed at the Lacombe site in 2011.

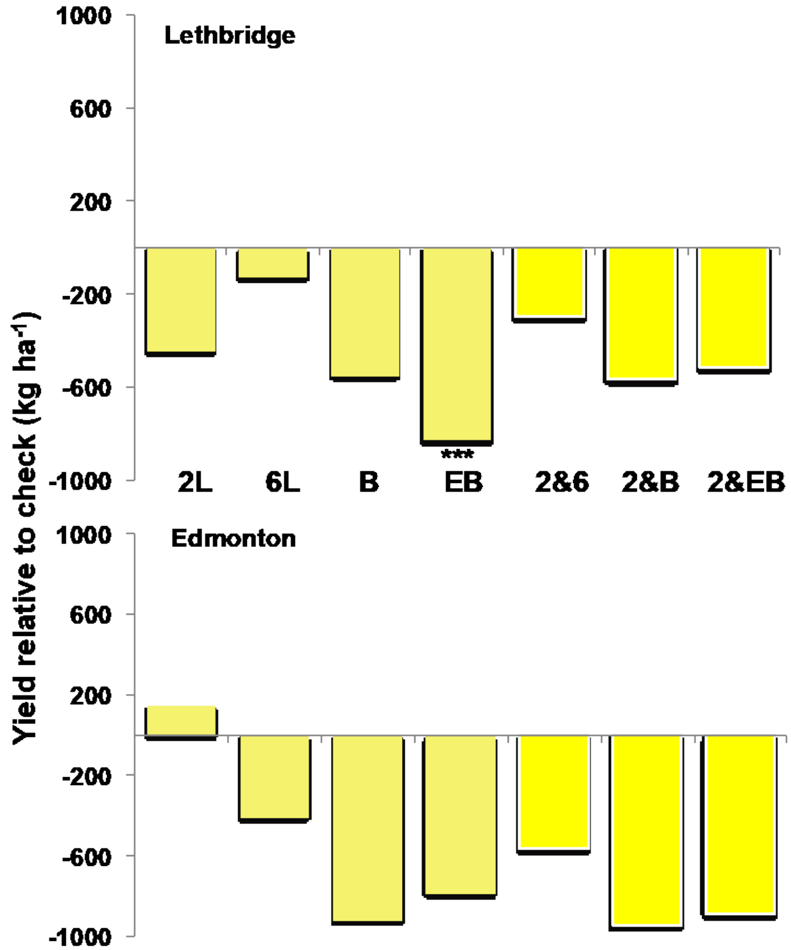

Figure 8. Response of glyphosate-resistant (RR) canola yield to late and sequential applications of glyphosate at Lethbridge and Edmonton, AB in 2010. Unsprayed check yields were 3811 kg ha^{-1} and 5059 kg ha^{-1} at Lethbridge and Edmonton, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

Some insight can be gained into the sizeable reductions in yield observed in this study by further examining yield components. Late and sequential applications of glyphosate generally resulted in significant reductions in seeds per pod at Lethbridge and Edmonton, especially when applications occurred at the bolt stage of crop development (Fig. 9). Plants that received late applications (off label past the six-leaf stage) formed between 2-3 fewer seeds per pod at both locations. In addition, plants receiving an application of glyphosate at the bolt stage aborted significantly more pods per plant at Edmonton (Fig. 10). Although not statistically different from the unsprayed check, plants receiving a late application of glyphosate at all other treatments beyond the six-leaf stage saw marked increases in aborted pods, ranging between five and fifteen aborted pods per plant. In contrast, reductions in yield at Lethbridge were due to a reduction in seeds per pod only (Fig. 9), and this reduction resulted in a significant increase in thousand seed weight at the bolt and 2L&B stages (Fig. 10). Because there were fewer seeds to fill, the plants in these treatments were able to fill the seeds to a larger weight.

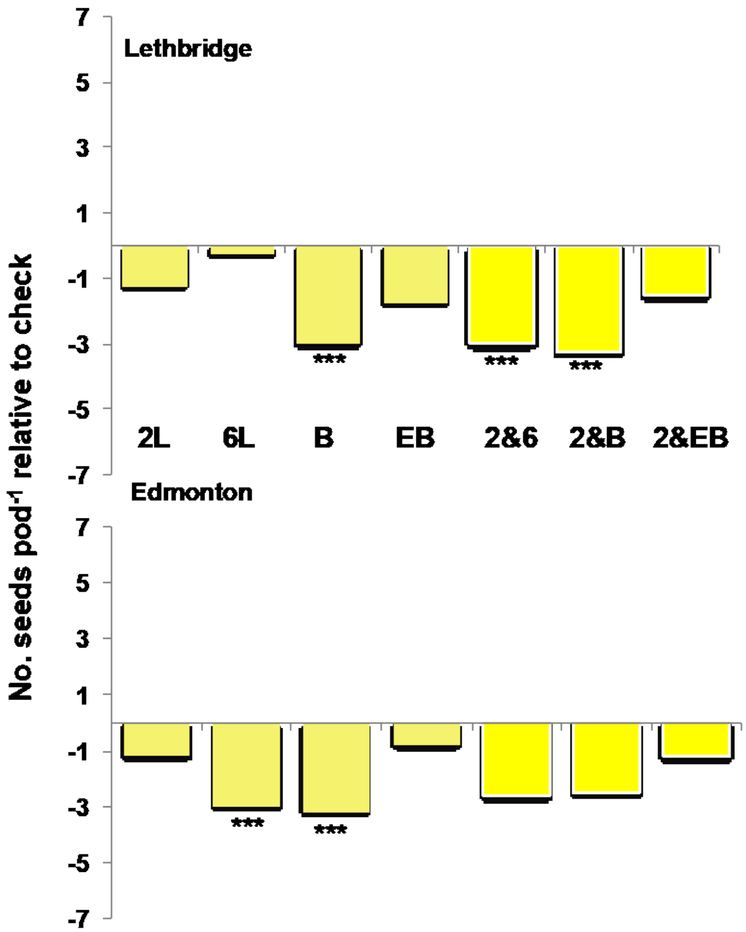

Figure 9. Seed production in glyphosate-resistant (RR) canola due to late and sequential applications of glyphosate at Lethbridge and Edmonton, AB in 2010. Seed production in the unsprayed check averaged 30 seeds pod^{-1} and 27 seeds pod^{-1}, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

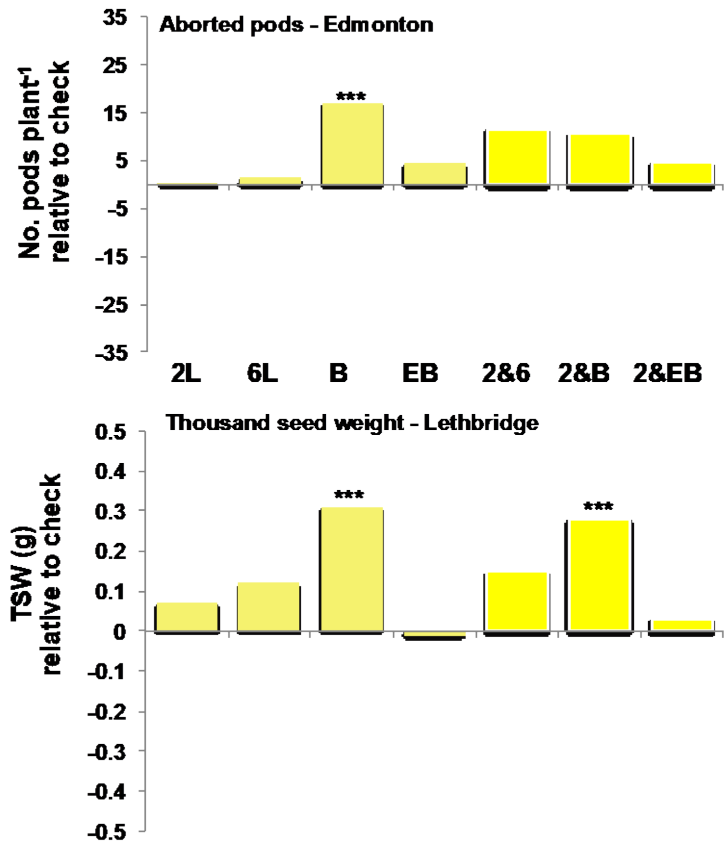

Figure 10. Pod abortion and thousand seed weight (TSW) in glyphosate-resistant (GLYR) canola due to late and sequential applications of glyphosate at Edmonton and Lethbridge, AB in 2011. Pod abortion and TSW in the unsprayed check averaged 57 pods $plant^{-1}$ and 4.0 g, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

All sites - 2010

Although differences between treatments were significant for yield at Lacombe, none of the treatments were significantly different than the control (Fig. 11). Interestingly, yield was always greater than the control if the herbicide was applied before the bolting stage and was lower when applied after. Although not significant, these values are very important because the EB yield reduction observed represents a loss of 7 bu/ac and about $63/ac at an assumed canola price of $10/bushel. Late application trends were similar at Edmonton, though all application timings had lower yields than the control. While yield reductions were minimal for single applications, sequential applications were reduced more substantially, with the 2&EB have a significantly lower yield than the control. This reduction equates to approximately 11 bu/ac and is a substantial economic decrease in gross revenue. It is important to point out that an application at the early bloom stage is not registered, but both a single and sequential application at the bolt stage are registered. The sequential application may need to be reconsidered as losses at both sites were consistent and sizeable, with reductions in gross revenue ranging between \$ 79-90 ha^{-1} was consistently lost.

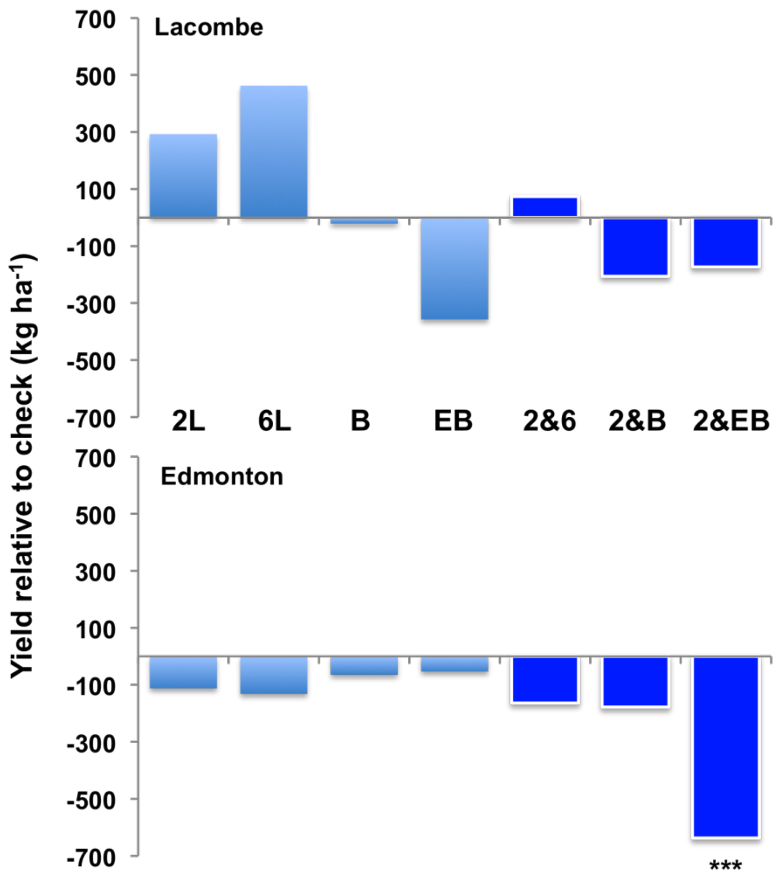

Figure 11. Response of glufosinate-resistant (LL) canola yield to late and sequential applications of glufosinate at Lacombe and Edmonton, AB in 2010. Unsprayed check yields were 5947 kg ha^{-1} and 6618 kg ha^{-1} at Lacombe and Edmonton, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

We can gain some insight as to why yield losses were so high at the Edmonton site when an application was made at the 2L&EB by looking at yield components. Fig. 12 clearly shows a significant increase in the number of aborted pods per plant when glufosinate was applied sequentially at the 2L&EB. This suggests that there may be problems with pollination when sequential applications are made at the 2L&EB and beyond and warrants further investigation, which will be conducted in our lab in the following two years. However, no significant differences were detected at Lacombe or among the other treatments at Edmonton. Moreover, none of the other yield components were affected by either late or sequential glufosinate applications. Based on this preliminary data, it appears that producers cannot make a glufosinate application past the bolt stage, and even sequential applications at the bolt stage have the potential to cause yield reductions.

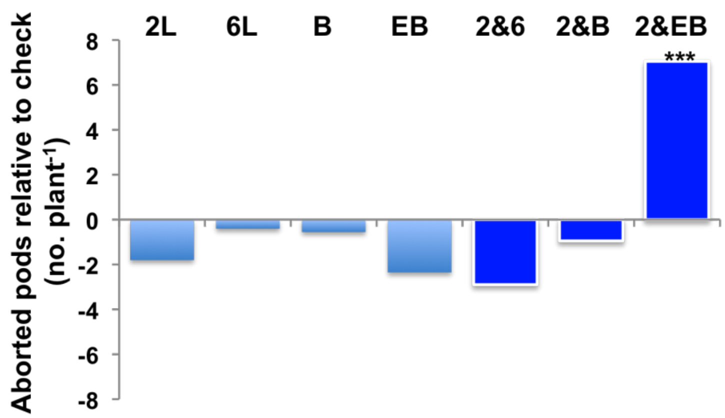

Figure 12. Pod abortion in glufosinate-resistant (LL) canola due to late and sequential applications of glufosinate at Edmonton, AB in 2010. Pod abortion in the unsprayed check averaged 10.8 pods plant^{-1}. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

With regard to the glyphosate-resistant system, little effect was observed when glyphosate was applied at the 2L and 6L stages, both of which are within the registered window for application (Fig. 13). Substantial reductions in yield were, however, observed at both sites when glyphosate was applied singly or sequentially at the early bloom stage. Reductions in yield at the early bloom stage varied from 210 kg ha^{-1} at Lacombe to nearly 600 kg ha^{-1} (10 bu/ac) at Edmonton when an application occurred at the 2L&EB. An interesting difference between sites was observed for applications at the bolt stage. Although they had almost no effect on yield at Lacombe, an application at the bolt stage in Edmonton proved to have a very adverse effect on canola yield components, with yield reductions in excess of 1000 kg ha^{-1} or 20 bu/ac. Once again, only the sequential application was significantly different from the control but at a $10/bu canola price, these reductions in yield could cost a grower $459 ha^{-1} or $185/ac in lost revenue. Thus, a substantial amount of income would be lost if a glyphosate application were made at the bolt stage of glyphosate-resistant canola. Although we are not yet sure of the reasons for the difference between sites, it is possible that moisture conditions could be to blame as more moisture was likely available to the developing crop at Lacombe as a function of it being seeded into fallow and not direct-seeded.

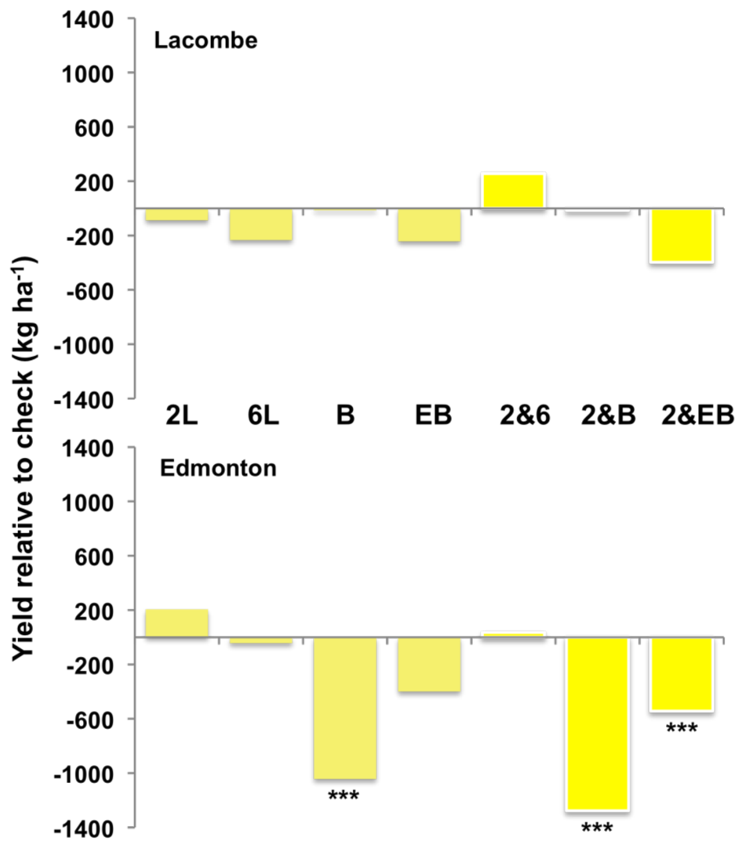

Figure 13. Response of glyphosate-resistant (RR) canola yield to late and sequential applications of glyphosate at Lacombe and Edmonton, AB in 2010. Unsprayed check yields were 4927 kg ha^{-1} and 5919 kg ha^{-1} at Lacombe and Edmonton, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

Some insight can be gained into the sizeable reductions in yield observed in this study by further examining yield components. Late and sequential applications of glyphosate at Lacombe did not significantly affect any of the yield components measured, which is not that surprising considering effects on yield were reduced as well (data not shown). However, Fig. 4 shows that at the Edmonton site, significant increases in the number of aborted pods were observed when glyphosate was applied beyond the 6L stage. Average pod abortion per plant in the late and sequential treatments ranged from 15 to nearly 35 pods per plant and represented 10 to 20% of total pod production. Moreover, it appears that the application of glyphosate at any stage reduced the number of seeds produced per pod at the Edmonton site (Fig. 14). However, we once again observed significant reductions when glyphosate was applied as a single application at the bolt and a multiple application after the bolting stage. In contrast, significant reductions in pods per plant and seeds per pod resulted in canola plants putting more resources into filling seeds and consequently, late and sequential applications produced larger seeds with significant increases in thousand seed weight ranging between 0.5 – 1.0 g per 1000 seeds (data not shown).

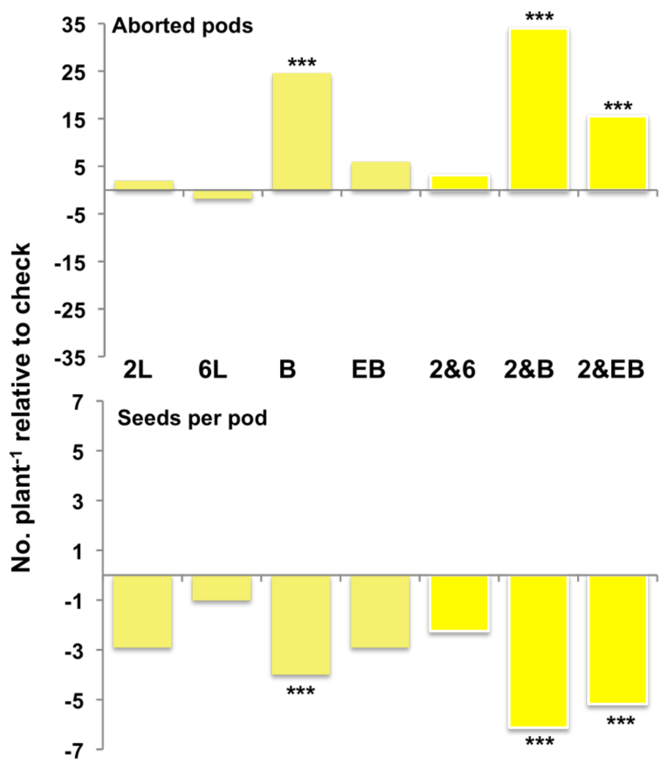

Figure 14. Pod abortion and seed production in glyphosate-resistant (RR) canola due to late and sequential applications of glyphosate at Edmonton, AB in 2010. Pod abortion and seed production in the unsprayed check averaged 10.8 pods plant^{-1} and 27 seeds pod^{-1}, respectively. Means were separated using Dunnett's test at ($P \leq 0.05$); *** denotes treatment is significantly different than the unsprayed check. 2L, two leaf; 6L, six-leaf; B, bolt; EB, early bloom.

Discussion

Results from the final year of this study show that late applications (past the 6-leaf stage) of glyphosate generally did not have a significant negative effect on yield, but economic impacts remain relevant. Some late application treatment combinations were significantly different from the control or from other groups of treatments (contrasts). These results concur with Schilling et al. (2006) who found that multiple sequential applications of glyphosate to GLYR canola produced significantly more injury than single applications. Similarly, Clayton et al. (2002) found that applications of glyphosate to GLYR canola between the one-leaf and four-leaf stage resulted in canola with the highest yields across most site-years. Site-years at Lacombe and Edmonton significantly benefited, in terms of yield, from sequential applications of glyphosate at later growth stages likely due to local weather favouring late emerging weeds. The result from the Clayton et al. (2002) study mirrors our findings that sequential applications of glyphosate often produced canola with the lowest yields at Saskatoon and Lethbridge.

On the other hand, Martin et al. (2001) reported that applications of glufosinate at the four-leaf to six-leaf stage produced canola with the highest yields when compared to other on-label herbicide timings. In general, sequential applications of glufosinate resulted in a decrease in yield in our study, which is in contrast to findings by Martin et al. (2001). These differences were observed within all the years of the study and suggest that applications of glufosinate at earlier (before 4-leaf) and later (after 6-leaf) stages can have a negative impact on the yield of GLUR canola. The differences reported between our study and that of Martin et al. (2001) may be due to the fact that weeds in our study generally were removed by hand to isolate the effects of the individual herbicides, whereas the crop was not kept weed-free in the Martin et al. (2001) study.

Other growth parameters in canola were also measured in this study. Seeds per pod, number of pods per plant, height, and 1000-seed weight were all recorded to investigate the impact of herbicide timing on factors other than yield. In general early application vs late applications and single applications vs sequential applications exhibited an influence on the number of seeds per pod, the number of pods per plant, and 1000-seed weight. Pline-Srnic et al. (2005) also found that late and sequential applications of Roundup at the 12-leaf and 4-8 leaf, respectively, significantly reduced the number of pods, number of seeds per pod, and total seed weight in cotton when compared to non-treated plants. Although not the same species, the applications of the same herbicide (glyphosate) produced similar results, suggesting that application timing of glyphosate may play a large role in influencing yield components. Our results are perhaps not surprising given that delayed herbicide applications to other HR crops (soybean, cotton, corn) caused reduced growth (Young et al., 2001; Pline et al., 2003; Norsworthy, 2004), altered reproductive morphology, male sterility, and reductions in seed set (Pline et al., 2002a,b; Thomas et al., 2004).

The lack of significant differences between site-years in the current study may be due to the effect of climatic variation on herbicide efficacy. Although not shown, unseasonal weather was present in the region during much the 2012 season, with moisture levels substantially higher than normal across much of the Prairies. It is possible that increased moisture and warm temperatures experienced in 2012 resulted in better metabolism of all herbicides utilized in this study, thereby resulting in less crop injury. Indeed, fewer effects were seen at Lacombe in 2010 and 2011 and Saskatoon in 2012, and this may also be associated with increased soil moisture availability on chem-fallow as opposed to other sites, which established the trial on cereal stubble. Because of the variability/ambiguity between sites with regards to effects on yield and yield components, we suggest that further research is needed, under controlled environment conditions, to pinpoint the conditions under which injury can result.

The lack of significant findings in the Clearfield (IMIR) system was generally consistent across most site-years of the study. This is peculiar as one would expect that significant effects would have been observed, particularly across the sequential treatments. We suspect that growers may be experiencing injury in this (IMIR) system when they are using Odyssey, which is a combination of imazamox + imazethapyr. It is possible that increased synergism results when the combination of these two herbicides is applied compared with when imazamox alone is applied, as was the case in the current study. Follow-up research should be directed at examining whether imazamox and imazethapyr applied alone have the same effect on canola crops if applied late compared with the combination of these two herbicides.

Conclusions

Based on these results, the response of canola to late and sequential applications is highly dependent on HR system. While we generally observed no significant effects on any variable measured in the IMIR system across eight site-years of data, applications made beyond the bolt and 6L stages in the GLUR and GLYR systems, respectively, have the potential to cause severe yield and economic losses. Despite the lack of differences in some site-years, trends across the other site-years are consistent and show that off-label applications in the GLYR system can have substantial impacts on crop yield, yield components, and even seed quality. It is clear that there is little flexibility in the GLYR system for late, off-label applications and these must be avoided whenever possible. We recommend that producers stay consistent with on-label applications of glyphosate as any off-label applications are risky. Nevertheless, producers are sometimes forced to make late applications due to inclement weather and in these circumstances, producers must weigh the perceived yield loss due to emerged weeds against the potential for sizeable reductions in yield before applying herbicides late in canola crops. Moreover, they must be aware that no recourse exists when herbicide applications are made off-label. However, more research needs to be done to identify thresholds at which yield loss due to weeds exceeds that caused by late herbicide applications.

Acknowledgements

The authors thank Alberta Innovates: BioIndustrial Solutions, the Alberta Canola Producers Commission, and Pioneer Hi-Bred for graciously funding the research. We also wish to thank Alberta Agriculture and Rural Development, Agriculture and Agri-Food Canada, the University of Alberta and the University of Saskatchewan for in-kind support including facilities and technical support staff. Finally, this work would not have been possible with the collective efforts of Susan Jess, Boris Henriquez, Larry Michielsen, Jennifer Zuidhof, Randall Brandt, Gerry Stuber, and Ken Sapsford.

Targets achieved compared to those contemplated

The following objectives were proposed for this project:

1. What is the response of HR canola to late and sequential herbicide applications with regard to reproductive ecology, yield, yield components?

   Achieved: This field experiment, conducted over 8 site-years, successfully identified that canola reproductive ecology does vary in response to late applications, though differences are a function of environmental variables.

2. Are there differences in crop tolerance among the different HR canola systems in several environments to develop recommendations specific to each HR canola system?

   Achieved: This field experiment, conducted over 8 site-years, showed that there are major differences in the response of HR canola systems to late applications. The GLYR system was most heavily and frequently impacted, GLUR canola was affected moderately and infrequently, and the IMIR system was generally not impacted. Environment remains a major variable impacting the effects within systems.

3. What are the cause(s) of any reductions in crop tolerance and are there practical solutions?

   Not achieved: We were unable to pinpoint the exact cause of the reductions in crop tolerance that we observed, particularly as they pertain to environmental variables. Anatomical and molecular (lab) studies isolating the mechanisms responsible for reproductive abnormalities and low yields were supposed to be completed. However, the PhD student working on this project failed out of both a PhD and MSc due to poor academic performance (failed courses). Coupled with a move by the PI to the University of Saskatchewan, it meant that this research was not completed. A portion of the money allocated for the lab work should have been refunded, to the best of my knowledge.

Extension and technology transfer activities to date:

2010

C. Willenborg, N. Harker, J. O'Donovan, R. Blackshaw. Evaluating the risks of late herbicide applications. Canola production field day. Lacombe, AB. July, 2010.

C. Willenborg, N. Harker, J. O'Donovan, R. Blackshaw. Late and sequential herbicide applications in herbicide-resistant canola systems. Alberta Agriculture/University of Alberta Crop Walk. St. Albert, AB. June 28 and July 27, 2010.

2011

Due to my move to Saskatchewan (joined the U of S as Assistant Professor) I was unable to show the plots at field days in AB in 2011. We also partnered with Pioneer Hi-Bred, our industry partner on the grant, who (with permission) shared portions of the results at several grower meetings/events.

2012

Harker, K.N., C.J. Willenborg, and R.E. Blackshaw. 2012. Sensitivity of Herbicide Resistant Canola to Late Herbicide Applications. Alberta Canola Industry Research Update. Edmonton, AB. April 12, 2012.

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