Weed Technology 2015 29:675–683

Late Glyphosate Applications Alter Yield and Yield Components in Glyphosate-Resistant Canola (*Brassica napus*)

Eric Tozzi, K. Neil Harker, Robert E. Blackshaw, John T. O'Donovan, Stephen E. Strelkov, and Christian J. Willenborg*

The development of glyphosate-resistant canola has provided improved weed-management options for growers, but crop tolerance to glyphosate may be inadequate at later growth stages. In this study, glyphosate-resistant canola 45H28 (RR) was used to determine the effects of glyphosate application timing on yield and yield components at several sites in western Canada. Canola received a single glyphosate applications at the two-leaf, six-leaf, bolting, and early bloom stages and sequential applications at the two-leaf + six-leaf, two-leaf + bolting, and two-leaf + early bloom stages. Contrasts were made between early vs. late, single vs. sequential, and on-label (two to six-leaf stage) vs. off-label (above six-leaf stage). In general, differences between application timings were observed for yield and yield components in 3 of 8 site-yr. Off-label applications of glyphosate (later than six-leaf) significantly decreased yield, seeds per pod, and increased thousand-seed weight and aborted pods in canola at the Lethbridge and St. Albert locations. Increased glyphosate translocation because of adequate, but not excessive, moisture to new growth may have suppressed new seed formation and encouraged pod abortion at the time of application in the 2010 and 2011 seasons. Results from this experiment demonstrate the importance of proper application timing of glyphosate on canola and can help better predict the effects of late applications.

Nomenclature: Glyphosate; canola, *Brassica napus* L.

Key words: Application timing, late, sequential, yield, seeds, pod, aborted.

El desarrollo de colza resistente a glyphosate ha brindado más opciones de manejo de malezas para los productores. En este estudio, se usó colza resistente a glyphosate 45H28 (RR) para determinar los efectos del momento de aplicación de glyphosate sobre el rendimiento y los componentes del rendimiento, en varios sitios en el oeste de Canada. La colza recibió una aplicación sencilla en los estadios de dos hojas, seis hojas, producción de tallo floral, y floración temprana y aplicaciones secuenciales en los estadios de dos hojas + seis hojas, dos hojas + producción de tallo floral, y dos hojas + floración temprana. Se realizaron contrastes entre las aplicaciones temprana vs. tardía, sencilla vs. secuencial, y dentro de las recomendaciones de la etiqueta (estadios de dos a seis hojas) vs. fuera de las recomendaciones de la etiqueta (después del estadio de seis hojas). En general, se vieron diferencias entre momentos de aplicación en el rendimiento y los componentes del rendimiento en 3 de los 8 sitios-años. Aplicaciones de glyphosate fuera de la etiqueta disminuyeron significativamente los rendimientos, el número de semillas por vaina, e incrementaron el peso de mil semillas y las vainas abortadas en colza en las localidades de Lethbridge y St. Albert. La precipitación excesiva podría haber causado una reducción en el rendimiento en la temporada 2012. Una translocación aumentada de glyphosate hacia tejidos de crecimiento nuevo, debido a una humedad adecuada, más no excesiva, podría haber prevenido la formación de semilla nueva y promovido el aborto de vainas al momento de aplicación en las temporadas 2010 y 2011. Los resultados de este experimento muestran la importancia de la aplicación de glyphosate en el momento adecuado en la colza y ayudan a predecir mejor los efectos de aplicaciones tardías.

The advent of herbicide-resistant (HR) canola has led to the ability to control a broad spectrum of weeds, which, in turn, has led to rapid adoption of the technology. Presently over 90% of canola varieties grown in western Canada are herbicide-resistant (Beckie et al. 2011). Glyphosate-resistant (GR) cultivars are very popular in western Canada and account for 44% of HR canola grown across western Canada (Smyth et al. 2011).

DOI: 10.1614/WT-D-15-00009.1

* First and sixth authors: Postdoctoral Scholar and Assistant Professor, Department of Plant Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada; second, fourth, and fifth authors: Adjunct Professor, Adjunct Professor, and Professor, Department of Agricultural, Life, and Environmental Sciences, University of Alberta 2-14 Agriculture Forestry Centre St., Albert, AB T6G 2P5, Canada; third author: Weed Scientist, Agriculture and Agri-Food Canada, 5403-1 Avenue South, Lethbridge, Alberta T1J 4B1, Canada. Corresponding author's E-mail: chris.willenborg@usask.ca

Ideally, herbicides should be applied to canola by the four-leaf stage to prevent yield loss from early emerging weeds (Clayton et al. 2002). Martin et al. (2001) observed that the greatest yields in GR canola occurred when applications of glufosinate were made between the four-leaf and six-leaf stages, indicating a critical period of weed control. However, herbicides applied too early may miss later-emerging weeds, which may then warrant further (sequential) herbicide applications (Schilling et al. 2006). In-crop glyphosate applications can be made until the six-leaf stage of GR canola at a maximum rate of 680 g ae ha^{-1} (Clayton et al. 2002). A second sequential application of glyphosate, however, can be made (after initial application of up to 450 g ha^{-1}) at a maximum rate of 450 g ha^{-1} if deemed necessary (SMA 2015). Additional constraints, such as adverse environmental conditions, can also force producers to apply herbicides at later-than-recommended growth stages, which could compromise crop tolerance and result in reductions in crop yield and quality.

In the past, producers and agronomists have reported canola pod abortion and potential reductions in GR canola yield, even under optimal environmental conditions (Anonymous 2006; Barker 2007). Questions have been raised regarding the tolerance of GR canola to a single, late application and to sequential applications of glyphosate. This is perhaps not surprising given that several GR crops commercialized to date have demonstrated sensitivity to glyphosate, ranging from leaf injury to reduced yields. Leaf injury (chlorosis) on GR soybean [*Glycine max* (L.) Merr.] treated with single or sequential applications of glyphosate, has been reported by Krausz and Young (2001). GR cotton (*Gossypium hirsutum* L.) treated with late (four-leaf and eight-leaf stage) glyphosate applications exhibited reproductive abnormalities, including inhibited elongation of the staminal column and filament, which increased the distance from the anthers to the receptive stigma tip during the first week of flowering (Pline et al. 2002b). The increased anther–stigma distance resulted in 42% less pollen deposited on the stigmas of glyphosate-treated plants than there was in nontreated plants. Pollen grains from glyphosate-treated plants also exhibited morphological abnormalities because of the inhibition of microgametogenesis (Pline et al. 2002b). Moreover, pollen viability was 51 and 38% lower for the first and second week of cotton flowering, respectively, in GR cotton plants treated with a four-leaf and eight-leaf POST treatment of glyphosate than it was in GR plants that were not treated (Pline et al. 2002a, 2003). The reproductive abnormalities observed in GR cotton (*Gossypium hirsutum* L.) resulted from significantly lower resistance-gene expression levels in the male reproductive tissue of cotton flowers (Pline et al. 2002b). Roundup Ready Flex Cotton (Monsanto Company, 800 N. Lindburgh Blvd., St. Louis, MO 63167) has since solved this issue (Main et al. 2007). Glyphosate has also been shown to induce male sterility in GR corn (*Zea mays* L.), which, although concerning for plant breeders, did not negatively affect pollination and seed set (Thomas et al. 2004). Baucom et al. (2008) reported similar increases in male sterility with glyphosate application to a GR tall morningglory [*Ipomoea purpurea* (L.) Roth] biotype.

In canola, Clayton et al. (2002) demonstrated that early glyphosate applications produced optimal GR canola yields, but yields were always compared with a weedy check. Nevertheless, their data showed a decrease (although inconsistent) in yield with late (six-leaf) glyphosate applications, but because plots were not kept weed-free, it was not possible to determine whether crop tolerance or weed competition was the cause of that decline. Schilling et al. (2006) reported that applying glyphosate to GR canola plants caused significant declines in biomass compared with untreated controls. However, it is uncertain whether that translated into reductions in yield and yield components. Despite several reports of glyphosate-tolerance problems in other GR crops (Pline et al. 2002a,b; Thomas et al. 2004), only anecdotal evidence exists to suggest that there may be similar problems in canola. Therefore, the objective of this study was to isolate any potential adverse effects of late or sequential glyphosate applications on GR canola yield and yield-components.

Materials and Methods

Experiments were conducted at Lacombe, Alberta, Canada (52.47°N, 113.74°W), from 2010 to 2012; St. Albert, Alberta (53.63°N, 113.62°W), from 2010 to 2011; Lethbridge, Alberta (49.68°N, 112.84°W), from 2011 to 2012; and at one location

Table 1. Field operations for preseed (trifluralin g ai ha^{-1}), seeding (seeds m^{-2}), glyphosate (g ae ha^{-1}), and insecticide (ai ha^{-1}) rates at Lacombe, AB, Canada, and St. Albert, AB, from 2010-2011, and Saskatoon, SK, Lethbridge, AB, and Lacombe, AB, in 2012.

Site–Year	Preseed (trifluralin)	Seeding	Glyphosate	Insecticide
	g ai ha^{-1}	seeds m^{-2}	g ae ha^{-1}	g ai ha^{-1}
Lacombe–2010	1,705	150	450	–
Lacombe–2011	1,705	150	450	–
Lacome–2012	1,705	150	450	Deltamethrin (6.2)
St. Albert–2010	1,705	150	450	–
St. Albert–2011	1,705	150	450	–
Saskatoon–2012	1,705	150	450	–
Lethbridge–2010	1,705	150	450	–
Lethbridge–2011	1,100	150	450	λ–Cyhalothrin (10.1)
Lethbridge–2012	1,100	150	450	λ–Cyhalothrin (10.1)

in Saskatoon, Saskatchewan, Canada (52.13°N, 106.64°W), in 2012. Soil at Lacombe and St. Albert was a black chernozem, whereas the Saskatoon and Lethbridge sites were located on a dark-brown chernozem. Experimental plots measuring 2 by 6 m were established at each site following either barley (*Hordeum vulgare* L.) stubble (St. Albert, Lethbridge) or fallow (Lacombe, Saskatoon). The experimental design was a randomized complete block with four replications of each glyphosate treatment.

Before plot establishment, trifluralin (Table 1) and glyphosate (900 g ha^{-1}) with bromoxynil (314 g ai ha^{-1}) were surface-applied in the fall and spring, respectively, to provide residual weed control at the Lethbridge location. Before plot establishment, trifluralin (1,705 g ai ha^{-1}) was applied as a PPI treatment in the spring to provide residual weed control at the Lacombe, St. Albert, and Saskatoon locations (Table 1). Insecticides were also applied to plots as needed (Table 1). At each site, 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.

At all locations, GR canola 45H28 (RR) was planted at a rate of 150 seeds m^{-2} (Table 1). This variety contains the CP4-EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene that codes for reduced inhibition of native EPSPS in the presence of glyphosate, as well as the glyphosate oxidoreductase (*GOX*) gene that confers increased glyphosate metabolism to the plant. Glyphosate was applied as a single application at the two-leaf (2L), six-leaf (6L), bolt (B), and the early bloom (EB) stages of canola; sequential applications were applied at the 2L and 6L, 2L and B, and 2L and EB; an unsprayed treatment was included as a control. A glyphosate rate of 450 g ae ha^{-1} in single applications and 900 g ae ha^{-1} (total) in sequential applications was used (Table 1). Plots were hand-weeded weekly to maintain weed-free plots.

Crop densities were determined by counting plants in two, 1-m rows, 3 to 4 wks after crop emergence. Yield component data collected included the number of fully formed and aborted pods per plant, as well as the number of seeds per pod. At physiological maturity, but before harvest, five random plants in each plot were cut at the soil surface, carefully removed from each plot, and the numbers of fully formed and aborted pods were 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. Plots were harvested with a small-plot combine, and canola yields were determined from the central six rows of each eight-row plot. Samples were then dried for 1 wk at room temperature (21 C) to a constant moisture (10%), 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 the thousand seed weight (TSW). Temperature (C) and precipitation (mm) were also recorded at each site (Table 2).

Statistical Analysis. All residuals were initially tested to ensure that they conformed to the assumptions of ANOVA, 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 a linear mixed-effects model (PROC MIXED) in SAS (SAS Institute 2013; SAS Institute Inc., 100 SAS Campus

Table 2. Mean temperature (C) and total precipitation (mm) at the various field sties.

	2010		2011		2012		2011	
	Lacombe						Lethbridge	
	Mean tempearture	Total precipitation	Mean tempearture	Total precipitation	Mean tempearture	Total precipitation	Mean tempearture	Total precipitation
April	6	13.3	7.3	11.6	2.3	42.5	7.3	50
May	10.4	20.1	11.9	38	9.9	121.7	11.9	50.5
June	12.6	115.8	15.4	119.1	14.5	161.9	15.7	100
July	15.5	63	18.1	102.8	19.1	52	19.9	72
August	13.1	111.7	15.5	119.3	13.2	64.9	17.3	13
September	9.4	37.9	11.7	77.7	10	42.5	13.2	24

Drive, Cary, NC 27513-2414). In the model, site–years and block were considered random terms, whereas application timing was considered a fixed effect. Analysis of covariance using a Wald's Z test was performed to determine whether random factors could be combined. Dunnett's test was used to simultaneously compare all treatments and their combinations to a single control treatment with differences in treatment effects declared significant at $P < 0.05$. Preplanned single degree of freedom contrasts were used to make specific comparisons of interest.

Results and Discussion

Yield. Because of interactions between sites and years, all data were analyzed as site–years (Table 3). Significant differences in yield were observed at the Lethbridge location in 2011 and 2012 and at the St. Albert location in 2010 (Table 4). Applications of glyphosate at St. Albert in 2010 resulted in a decreased yield of 19% when applied at the 2L and B stage, 16% at the B stage, and 8% at the 2L and EB stage when compared with the unsprayed control. Similarly, applications of glyphosate at the EB stage at Lethbridge in 2011 resulted in a decreased yield of 35% when compared with the unsprayed control, whereas a yield reduction of 16% resulted from an application at the 2L and 6L stage when compared with the unsprayed control at Lethbridge in 2012.

Contrasts also exhibited differences when comparing groups of treatments (Table 5). At the Lethbridge location, differences were observed within On label vs. Control (–552 kg ha^{-1}) and Sequential early vs. Control (–817 kg ha^{-1}) in the glyphosate treatments (Table 5). At the Lacombe location, differences in yield were only observed with the Sequential late vs. Control (+448 kg ha^{-1}) in the glyphosate treatments (Table 5). The remaining sites-by-application timings did not result in reduced canola yield compared with untreated control plots, suggesting that sequential glyphosate applications did not result in reduced yield, possibly because of excessive moisture from local weather conditions at the time.

At Lethbridge in 2011, a significant reduction in yield relative to the unsprayed check was observed when a single application was made at the EB stage (Table 4). At St. Albert in 2011, a statistically significant reduction in yield relative to the check did not occur, but single and sequential applications made at the B stage and beyond resulted in a substantial reduction in yield (8 to 19%) relative to an application made at the 2L stage (Table 4). Reductions in yield at the B stage varied from 14% at Lethbridge (2011) to 18% at St. Albert (2011), with similar reductions occurring at the 2L and B (Table 4). These reductions are critical to growers as they would result in reduced revenue at St. Albert in 2011 ranging from \$457 ha^{-1} CDN (\$369 USD) with an application made at the B stage to \$237 ha^{-1} CDN (\$191 USD) when an application was made at the 2L and EB (Table 4). Thus, a substantial amount of income would be lost if a glyphosate application were made past the B stage of GR canola.

Results of this study show that, although late applications (past the 6L stage) of glyphosate only had a significant effect on yield in some site–years, a general trend of reduced yields was observed when applications were made beyond this point in almost all site–years. These results concur with Schilling et al. (2006), who found that multiple sequential

Table 2. Extended.

2012		2010		2011		2012	
Lethbridge		St. Albert				Saskatoon	
Mean tempearture	Total precipitation	Mean temperature	Total precipitation	Mean precipitation	Total precipitation	Mean precipitation	Total precipitation
4.9	50.5	5.6	41	1.2	19.2	5.3	36.9
11.4	84.8	8.1	105.9	11.2	15.6	10.1	150.1
15.3	19.7	13.9	79.4	13.6	128.2	15.8	113
22	1	15.6	146.7	15.3	150.2	19.7	90.6
16.9	17.5	14.4	38.8	14.6	10.8	17.3	66
12	22.1	8.2	40.5	11.9	5.2	12.8	21.2

applications of glyphosate to GR canola produced significantly more injury than single applications under greenhouse conditions. Similarly, Clayton et al. (2002) reported that applications of glyphosate to GR canola between the one-leaf and four-leaf stage resulted in canola with the highest yields across most site–years compared with late applications.

The inconsistent results between site and years in the current study may be due to the effect of climatic variation on herbicide efficacy and crop production. Unseasonal weather was present in the region during much of the 2012 season, with moisture levels substantially greater than normal across much of the Canadian prairies (Table 2). It is possible that excessive moisture experienced in 2012 resulted suboptimal growing conditions, resulting in lower than average yields (Table 4). The negative effects of excessive moisture may have outweighed any negative effects of late or sequential glyphosate applications. Fewer differences were observed at Lacombe in 2010 and 2011, which may be associated with increased, but not excessive, soil-moisture availability on chem-fallowed areas, as opposed to cereal stubble. The significant results observed at the St. Albert location in 2010 may have been due to increased moisture content near the soil surface because of direct seeding into standing stubble. Trials at Saskatoon and Lacombe locations were seeded into fallowed plots. Plots on chem-fallow would have allowed for greater relative moisture retention in the soil, thereby, providing greater metabolism of glyphosate after application in years where excessive moisture was not a concern (Din et al. 2011). The differences in moisture availability between sites may have been the cause of significant differences in yield and yield components found in this study. Because of the large variability in yield and yield components between sites, we suggest that further research is needed, under controlled environment conditions, to determine the conditions leading to canola injury.

Yield Components. Differences in the number of seeds per pod were observed at St. Albert in 2010 and 2011 as well as at Lethbridge in 2011 (Table 6). Reductions in seeds per pod from applications of glyphosate occurred with applications made at the B (15%), 2L and B (23%), and 2L and EB (19%) stages at St. Albert in 2010 and at the B (12%) and 6L (11%) stages at St. Albert in 2011 (Table 6). Likewise, a reduction in seeds per pod from applications of glyphosate was observed at the B (10%), 2L and B (11%), and 2L and 6L (10%) stages at Lethbridge in 2011 (Table 6).

Table 3. P values derived from ANOVA of glyphosate-resistant canola yield, number of seeds per pod, number of aborted pods, and thousand-seed weight (TSW [g]). An asterisk (*) denotes significance at $P \leq 0.05$.

Source	Yield	Seeds pod^{-1}	Aborted pods	TSW
		P values		
Site–year	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
System timing	0.9063	0.0001*	0.0105*	0.1228
Block	0.9896	0.1757	0.4863	0.9402
Site–year × system timing	0.9615	0.875	0.2004	0.6427

https://doi.org/10.1614/WT-D-15-00009.1 Published online by Cambridge University Press

Table 4. Response of glyphosate-resistant canola yield from early/late and sequential applications of glyphosate at Lacombe, AB, and St. Albert, AB, from 2010–2011 and Saskatoon, SK, Lethbridge, AB, and Lacombe, AB, in 2012. Means were separated using Dunnett's test at ($P \leq 0.05$) and expressed relative to control means.[a,b]

Stage	St. Albert–2010	Lacombe–2010	St. Albert–2011	Lacombe–2011	Lethbridge–2011	Saskatoon–2012	Lacombe–2012	Lethbridge–2012
	kg ha^{-1}							
2L	205.5	−88.1	145.1	193.2	−443.7	−11	461.6	−560.9
2L and 6L	35.6	256.5	−564.8	676.4	−297	−58.3	461.4	−817.2*
2L and B	−1,277.4*	−15.1	−943.8	558.7	−562.5	159.2	342.5	−213
2L and EB	−543.9*	−397.1	−888.4	205.4	−514.7	100.7	554.4	−581.4
6L	−43.4	−223.6	−408.5	489.6	−127.5	−126	194.5	−187.9
B	−1,043.5*	−14.1	−921.0	309.7	−551.9	39.2	301.3	−185.8
EB	−399.2	−242.9	−791.1	71.4	−829.7*	173.2	330.4	−308.2
LSD	−996	−835	−948	−1,217	−813	−732	−669	−634
Control	6,618.1	5,949.5	5,059.8	6,246	3,811.6	2,341.2	2,638.6	4,957.7

[a] Abbreviations: 2L, two-leaf stage; 6L, six-leaf stage; B, bolt; EB, early bloom.

[b] An asterisk (*) denotes treatment is significantly different from the control.

Differences in the number of aborted pods were also observed at the St. Albert site in 2010 and 2011 (Table 7) and at the Lethbridge site in 2012 (Table 7). At St. Albert, a significant increase in aborted pods was observed when herbicide applications were made at the B (124%), 2L and B (210%), and 2L and EB (50%) stages in 2010 and a decrease was observed at the B (14%) stage in 2011 (Table 7). At the Lethbridge site in 2012, applications of glyphosate resulted in increased aborted pods across all growth stages and an increase at the 2L and EB (72%) stage (Table 7).

Average pod abortion per plant in the late and sequential treatments ranged from 15 to nearly 35 pods plant^{-1} and represented as much as 10 to 20% of total pod production in some site–years (Table 7). Plants that received late applications (off label past the 6L stage) formed two to three fewer seeds per pod at the St. Albert and Lethbridge locations. In addition, plants receiving an application of glyphosate at the B stage aborted more pods per plant at St. Albert (Table 7). Although not statistically different from the unsprayed check in a few site–years, plants receiving a late application of glyphosate at all other stages beyond the 6L stage saw marked increases in aborted pods, ranging between 5 and 15 aborted pods plant^{-1} (Table 7). Moreover, it appears that the application of glyphosate at any stage reduced the number of seeds produced per pod at the St. Albert site when compared with the control (Table 7). In general, the number of seeds per pod and the number of aborted pods per plant were reduced when glyphosate was applied as a late application or sequential application. This resulted in the production of plants with

Table 5. Single degrees-of-freedom contrasts and the associated estimates of the difference between yield means in glyphosate-resistant canola at Lacombe, AB, and St. Albert, AB, from 2010–2011 and at Saskatoon, SK, Lethbridge, AB, and Lacombe, AB, Canada, in 2012.[a]

	Lacombe–2010	St. Albert–2010	Lethbridge–2011	Lacombe–2011	St. Albert–2011	Lethbridge–2012	Saskatoon–2012	Lacombe–2012
	kg ha^{-1}							
On-label vs. control	−18.4	66.0	−289.4	453.1	−276.1	−522.0*	−65.1	372.5
Off-label vs. control	−167.3	−816.0	−614.7	286.3	−886.1	−322.1	−35.0	382.2
Single vs. Sequential	−90.3	275.1	−30.2	−214.2	305.1	226.5	155.8	−130.8
Single, early vs. control	−155.9	81.1	−285.6	341.5	−131.8	−374.4	−68.5	328.1
Single, late vs. control	−128.5	−721.4	−690.9	190.6	−856.1	−247.0	106.6	315.9
Sequential, early vs. control	256.5	35.7	−297.0	676.5	−564.9	−817.3*	−58.6	461.4
Sequential, late vs. control	−206.1	−910.6	−538.6	382.1	−916.1	−397.2	−176.3	448.5*

[a] An asterisk (*) denotes statistically significant differences between comparisons at $P < 0.05$.

https://doi.org/10.1614/WT-D-15-00009.1 Published online by Cambridge University Press

Table 6. Response of glyphosate-resistant canola seeds per pod from early to late and sequential applications of glyphosate at Lacombe, AB, and St. Albert, AB, from 2010–2011 and Lethbridge, AB, and Lacombe, AB, Canada in 2012. Means were separated using Dunnett's test at ($P \leq 0.05$ and expressed relative to control means).[a,b]

Stage	St. Albert–2010	Lacombe–2010	St. Albert–2011	Lacombe–2011	Lethbridge–2011	Lacombe–2012	Lethbridge–2012
	seeds pod^{-1}						
2L	−2.91	0	−1.175	−0.67	−1.25	−0.57	0.25
2L and 6L	−2.25	0	−2.6	0.16	−3*	−1.78	0
2L and B	−6.13*	−1.25	−2.475	−1.02	−3.25*	−0.41	−0.75
2L and EB	−5.18*	−0.5	−1.225	−2.57	−1.5	−0.77	−1
6L	−1.02	−1.5	−3.075*	−0.78	−0.25	−1.06	0.25
B	−4*	0.25	−3.225*	−1.71	−3*	−1.04	−0.75
EB	−2.91	−1.25	−0.8	−1.73	−1.75	−0.05	−0.75
LSD	−3.98	−2.11	−2.82	−3.07	−2.98	−2.19	−2.64
Control	26.85	26.25	26.6	29.59	30	27.51	29.25

[a] Abbreviations: 2L, two-leaf stage; 6L, six-leaf stage; B, bolt; EB, early bloom.
[b] An asterisk (*) denotes treatment is significantly different from the control.

fewer pods with less seed, although the seeds produced were of an increased size (Table 8).

Late and sequential applications of glyphosate in some site–years produced larger seeds with increases in TSW ranging from 0.5 to 1.0 g per 1,000 seeds (Table 8). The St. Albert site in 2010 displayed significant increases in TSW when glyphosate applications were made at the B (29%), EB (15%), 2L and EB (31%), and 2L and B (13%) stages. Likewise, applications made at the B (11%) and 2L and B (9%) stages at Lethbridge in 2011 exhibited increases when compared with the unsprayed control (Table 8). Most notably, individual and sequential applications at the B and EB stages resulted in the largest increase when compared with all other stages in any of the site–years in which this study was carried out (Table 8).

The results of this study indicate that early application vs. late applications of glyphosate, and single applications vs. sequential applications of glyphosate influence GR canola yield via reductions in the number of seeds per pod, the number of pods per plant, and alterations to TSW. Pline-Srnic et al. (2005) also found that late and sequential applications of glyphosate at the 12-leaf and 4- to 8-leaf stages, respectively, significantly reduced the number of pods and seeds per pod and increased total seed weight in cotton when compared with nontreated plants. Pline-Srnic et al. (2005) found that glyphosate applied sequentially at 12-leaf and at 4-

Table 7. Response of the aborted pods from glyphosate-resistant canola from early to late and sequential applications of glyphosate at Lacombe, AB, and St. Albert, AB, from 2010–2011, and Lethbridge, AB, and Lacombe, AB, Canada in 2012. Means were separated using Dunnett's test at ($P \leq 0.05$) and expressed relative to control means.

Stage	St. Albert–2010	Lacombe–2010	St. Albert–2011	Lacombe–2011	Lethbridge–2011	Lacombe–2012	Lethbridge–2012
	pods						
2L	1.95	0.9	0.5	−4.4	−6.3	−1.1	17
2L and 6L	3.2	2.4	11.5	−3.5	−9.0	−1.9	28.3
2L and B	34.0*	2.1	10.6	−1.2	−3.5	0.5	25.3
2L and EB	15.6*	2.5	4.6	−1.1	6.0	5.1	58.8*
6L	−1.85	1.4	1.3	−0.8	−8.3	−0.5	8.8
B	24.6*	4.1	16.9*	−0.1	0.8	−0.9	20.6
EB	5.9	4.8	4.6	0.0	−8.5	4.7	1.5
LSD	−15.4	−7.2	−12.1	−7.3	−30.9	−8.6	−50.3
Control	11.1	11.9	19.6	12.4	56.8	6.3	81.5

[a] Abbreviations: 2L, two-leaf stage; 6L, six-leaf stage; B, bolt; EB, early bloom.
[b] An asterisk (*) denotes treatment is significantly different from the control.

Table 8. Response of glyphosate-resistant canola thousand-seed weight from early/late and sequential applications of glyphosate at Lacombe, AB and St. Albert, AB from 2010–2011 and Saskatoon, SK, Lethbridge, AB and Lacombe, AB, Canada, in 2012. Means were separated using Dunnett's test at ($P \leq 0.05$) and expressed relative to control means.[a,b]

Stage	St. Albert–2010	Lacombe–2010	Edmonton–2011	Lacombe–2011	Lethbridge–2011	Saskatoon–2012	Lacombe–2012	Lethbridge-2012
	g thousand seeds^{-1}							
2L	−0.01	−0.02	−0.02	−0.08	0.07	−0.19	−0.03	−0.18
2L and 6L	−0.03	0.05	−0.06	−0.08	0.15	−0.17	0.10	−0.05
2L and B	1.18*	−0.06	−0.04	0.07	0.27*	−0.16	0.19	0.17
2L and EB	0.49*	−0.07	0.01	−0.01	0.03	−0.1	0.01	0.22
6L	0.02	0.08	0.17	−0.86	0.12	−0.1	0.03	0.03
B	1.12*	−0.07	−0.09	0.04	0.31*	−0.07	0.06	0.11
EB	0.57*	−0.08	0.06	−0.08	−0.01	−0.02	0.01	0.05
LSD	−0.36	−0.21	−0.41	−0.33	−0.26	−1.27	−0.30	−0.27
Control	3.80	2.31	3.95	4.00	2.99	3.55	3.73	3.65

[a] Abbreviations: 2L, two-leaf stage; 6L, six-leaf stage; B, bolt; EB, early bloom.

[b] An asterisk (*) denotes treatment is significantly different from the control.

to 8-leaf stages reduced cumulative flower production after 8 wk by 65 and 54%, respectively. Similarly, Pline et al. (2002c) reported that applications of glyphosate to GR cotton at the four- and eight-leaf stage inhibited the growth of the staminal column and filament, which increased the distance from the anthers to the receptive stigma tip by 4.9 to 5.7 mm during the first week of flowering. This resulted in 42% less pollen being deposited, potentially resulting in yield loss at the time of harvest. Although the current study did not focus on anatomical changes associated with late glyphosate applications in canola, it is possible that the increased distance between the anthers and stigma is the causal mechanism behind the differences observed in the current study. In addition, Pline et al. (2002a) found that applications of glyphosate to GR cotton at the four- and eight-leaf stage reduced pollen viability and seed set. The production of aborted pollen, pollen with reduced viability, or poor pollen deposition on the stigma in treatments receiving late glyphosate applications (Pline et al. 2002b) may have contributed to the reduced yield and negative effects on yield components that were observed in this experiment. Further research is needed to identify the mechanism responsible for the reductions in GR canola yield and yield components reported in this study.

The results of the current study suggest that glyphosate applications made beyond the 6L stage in GR systems 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 were consistent and show that off-label (beyond 6L) applications in the GR system can have substantial effects on crop yield and yield components, resulting in significant declines in the income derived from GR canola crops. Our results are perhaps not surprising given that delayed herbicide applications to other HR crops (soybean, cotton, corn) caused reduced growth (Norsworthy 2004; Pline et al. 2003; Young et al. 2001), altered reproductive morphology, male sterility, and reductions in seed set (Pline et al. 2002a,b; Thomas et al. 2004).

Our data show reduced tolerance in the GR system for late/off-label applications, and these must be avoided whenever possible. Variability between site-years suggests that more-detailed research needs to be conducted to determine the causes of canola injury that we observed. It is crucial that producers make on-label applications of glyphosate because any off-label applications may be detrimental to crop production. Nevertheless, growers are sometimes forced to make late applications because of inclement weather, and in these circumstances, they must weigh the perceived yield loss from emerged weeds against the potential for sizeable reductions in yield before applying herbicides at growth stages beyond the 6L in GR 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 from weeds exceeds that caused by late herbicide applications.

Acknowledgments

The authors thank Alberta Innovates: Bio Industrial 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, Louis Molnar, Randall Brandt, Gerry Stuber, and Ken Sapsford.

Literature Cited

Anonymous (2006) CanolaFact: Missing Pods or Blanks on the Main Stem! What Could Be the Cause? Winnipeg, MB: Canola Council of Canada. 5 pp

Barker B (2007) Canola pod abortion investigated. Top Crop Manager 34:16–17

Baucom RS, Mauricio R (2008) Constraints on the evolution of tolerance to herbicide in the common morning glory: resistance and tolerance are mutually exclusive. Evolution 62:2842–2854

Beckie HJ, Harker KN, Légère A, Morrison MJ, Séguin-Swartz G, Falk KC (2011) GM Canola: the Canadian experience. Farm Policy J 8:43–49

Clayton GW, Harker KN, O'Donovan JT, Baig MN, Kidnie MJ (2002) Glyphosate timing and tillage system effects on glyphosate-resistant canola (*Brassica napus*). Weed Technol 16:124–130

Din J, Khan SU, Ali I, Gurmani AR (2011) Physiological and agronomic response of canola varieties to drought stress. J Anim Plant Sci 21:78–82

Krausz RF and Young BG (2001) Response of glyphosate-resistant soybean (*Glycine max*) to trimethylsulfonium and isopropylamine salts of glyphosate. Weed Technol 15:745–749

Main CL, Jones MA, Murdock EC (2007).Weed response and tolerance of enhanced glyphosate-resistant cotton to glyphosate. J Cotton Sci 11:104–109

Martin SG, Van Acker RC, Friesen LF (2001) Critical period of weed control in spring canola. Weed Sci 49:326–333

Norsworthy JK (2004) Tolerance of a glyphosate-resistant soybean to late-season glyphosate applications. Weed Technol 18:454–457

Pline WA, Edmisten KL, Oliver T, Wilcut JW, Wells R, Allen HS (2002a) Use of digital image analysis, viability stains, and germination assays to estimate conventional and glyphosate-resistant cotton pollen viability. Crop Sci 42:2193–2200

Pline WA, Viator R, Wilcut JW, Edmisten KL, Thomas J, Wells R (2002b) Reproductive abnormalities in glyphosate-resistant cotton caused by lower CP4-EPSPS levels in the male reproductive tissue. Weed Sci 50:438–447

Pline WA, Wilcut JW, Duke SO, Edmisten KL, Wells R (2002c) Tolerance and accumulation of shikimic acid in response to glyphosate applications in glyphosate-resistant and conventional cotton (*Gossypium hirsutum* L.). J Agric Food Chem 50:506–512

Pline WA, Edmisten KL, Wilcut JW, Wells R, Thomas J (2003) Glyphosate-induced reductions in pollen viability and seed set in glyphosate-resistant cotton and attempted remediation by gibberellic acid (GA_3). Weed Sci 51:19–27

Pline-Srnić WA, Thomas WE, Viator RP, Wilcut JW (2005) Effects of glyphosate application timing and rate on sicklepod (*Senna obtusifolia*) fecundity. Weed Sci 19:55–61

SAS Institute (2013) SAS User's Guide. Version 9.3. Cary, NC: SAS Institute

[SMA] Saskatchewan Ministry of Agriculture (2015) Guide to Crop Protection. 554 p

Schilling BS, Harker KN, King JR (2006) Glyphosate can reduce glyphosate-resistant canola growth after individual or sequential applications. Weed Technol 20:825–830

Smyth SJ, Gusta M, Belcher K, Phillips PBW, Castle D (2011) Changes in herbicide use after adoption of HR canola in western Canada. Weed Technol 25:492–500

Thomas WE, Pline-Srnić WA, Thomas JF, Edmisten KL, Wells R, Wilcut JW (2004) Glyphosate negatively affects pollen viability but not pollination and seed set in glyphosate-resistant corn. Weed Sci 52:725–734

Young BG, Young JM, Gonzini LC, Hart SE, Wax LM, Kapusta G (2001) Weed management in narrow- and wide-row glyphosate resistant soybean (*Glycine max*). Weed Technol 15:112–12

Received January 19, 2015, and approved June 7, 2015.

Associate Editor for this paper: Darren Robinson, University of Guelph.