Control of Gene-Stacked Canola by Alternative Herbicides

H. J. Beckie[1], G. Séguin-Swartz[1], H. Nair[1], S. I. Warwick[2], and E. Johnson[1]

[1]Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, S7N 0X2
[2]Eastern Cereal and Oilseeds Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, K1A 0C6

Key words: *Brassica napus*, gene stacking, herbicide resistance, pleiotropic effects, volunteers

Abstract

Unintentional herbicide resistance gene stacking in canola may alter the sensitivity of volunteers to herbicides of alternative modes of action commonly used for their control. Greenhouse experiments were conducted to investigate the dose response of three single herbicide-resistant (HR) cultivars (glyphosate, glufosinate, imidazolinone), one non-HR cultivar, and seven multiple (double or triple) HR experimental lines treated at the two- to three-leaf stage to 2,4-D (amine and ester), MCPA ester, and metribuzin; and of one non-HR and four HR cultivars (glyphosate, glufosinate, imidazolinone, bromoxynil) to 2,4-D amine applied at two growth stages (two to three, and five to six leaves). All canola cultivars or lines treated at the two- to three-leaf stage responded similarly to increasing doses of the three herbicides. At the five- to six-leaf stage, however, the bromoxynil HR cultivar was less sensitive to 2,4-D than the other cultivars. The results of this study suggest that canola with multiple herbicide resistance traits does not differ from cultivars that are non-HR or single HR in its sensitivity to herbicides commonly used to control volunteers. All volunteers, whether non-HR, single HR, or multiple HR, should be treated when plants are most sensitive to herbicides (two- to four-leaf stage) to reduce their interference against crops and their perpetuation of gene flow.

Introduction

Pollen flow between *B. napus* canola cultivars with different HR traits resulted in volunteers with multiple resistance at a field site in western Canada (Hall et al. 2000). In 1997 in northern Alberta, a field of glyphosate HR canola was grown adjacent to a field of glufosinate HR and imidazolinone HR canola. Volunteers were selected with glyphosate in 1998. These volunteers flowered and produced seeds that contained individuals resistant to glyphosate and glufosinate, glyphosate and imazethapyr, and glyphosate, imazethapyr, and glufosinate. Two triple HR individuals were detected, with one plant located 550 m from the glyphosate HR pollen source. More recently, a study at 11 sites in Saskatchewan, Canada where glyphosate HR *B. napus* canola was grown adjacent to glufosinate HR *B. napus* canola documented gene flow to the limits of the study areas – a maximum distance of 800 m – based on occurrence of double HR progeny of parental plants (Beckie et al. 2001) and double HR volunteers (Beckie et al., unpubl. data). The results of both studies suggest that HR gene stacking may be common in *B. napus* canola volunteers in western Canada.

Cultural or mechanical practices that are recommended to farmers to manage multiple HR canola volunteers include (Thomas 2001): (1) leaving seeds on or near the soil surface as long as possible after harvest because a high percentage will germinate in the fall and be killed by frost (Légère et al. 2001), whereas seeds incorporated into the soil may develop secondary dormancy that will increase persistence (Gulden et al. 2002; Pekrun et al. 1998); (2) using tillage immediately prior to seeding; (3) silaging and green manuring to prevent seed set in volunteers; (4) isolating fields of canola with different herbicide resistance traits to reduce outcrossing; (5) following canola with a cereal crop and rotating canola in a 4-yr (minimum) diverse cropping sequence to deplete volunteers from the seedbank over time (which also facilitates use of alternative herbicides and herbicide rotation), and growing competitive crops to minimize volunteer canola interference (by choice of species and manipulation of agronomic practices such as higher seeding rates and precision fertilizer placement); (6) scouting fields for volunteers not controlled by weed management treatments and preventing seed set; (7) using pedigreed seed to reduce the probability of the presence of off-types with different herbicide resistance traits; and (8) reducing seed loss during harvest by swathing at the correct crop development stage and properly adjusting combine settings. In a study in Saskatchewan in 1999 and 2000, average *B. napus* seed losses of 5.9% of crop yield (3,000 viable seeds m^{-2}) was measured in 35 farmers' fields (Gulden et al. 2003); yield losses among farmers ranged from 3.3 to 9.9% or 9- to 56-times the recommended seeding rate of canola.

Herbicides, however, will remain the dominant weed control tool for managing multiple HR canola volunteers. Those volunteers possessing glyphosate resistance are most likely to be noticed because glyphosate is used for pre-seeding burnoff and chem-fallow, whereas glufosinate and Group 2 herbicides are not. Glufosinate is used only in glufosinate HR canola. Volunteers possessing imidazolinone resistance may remain in cereals or field pea where only Group 2 herbicides are applied. Herbicides with alternative modes of action, such as phenoxy herbicides (Group 4, e.g., 2,4-D, MCPA) or photosystem II inhibitors (Group 5, e.g., metribuzin), are generally recommended (alone or in a mixture) to control canola volunteers with single or stacked herbicide resistance traits. For example, glyphosate can be mixed with 2,4-D to control volunteers prior to seeding a cereal crop that follows glyphosate HR canola in rotation. Little research, however, has been conducted to investigate the possibility of altered herbicide sensitivity of such plants to herbicides of alternative modes of action due to the genetic transformation (i.e., pleiotropic effects). In the UK, double HR (glyphosate and glufosinate) winter and spring lines of *B. napus* developed by traditional plant breeding were found to be equally susceptible to paraquat (Group 22), metsulfuron (Group 2), or mecoprop (Group 4) as single HR and non-HR lines (Senior et al. 2002). In this greenhouse study reported herein, we examine: (1) the response of four non-HR or single HR canola cultivars, and seven multiple (double or triple) HR experimental lines to 2,4-D, MCPA, and metribuzin; and (2) the response of non-HR and single HR cultivars to 2,4-D when applied at two plant growth stages.

Materials and Methods

Response to Alternative Herbicides

The *B. napus* cultivar 'Westar' was the non-HR check used in the study; '45A50', 'SW Legion LL', and '45A71' were the glyphosate HR, glufosinate HR, and imidazolinone HR cultivars, respectively. The seven experimental homozygous lines with double (2HR) or triple resistance traits (3HR) were developed by traditional breeding. The cultivars used in the crosses were '45A51' (glyphosate HR), 'SW Legion LL', and '45A71'. The 2HR lines were IL (imidazolinone/glufosinate), IR (imidazolinone/glyphosate), LR (glufosinate/glyphosate), and two lines of RL (glyphosate/glufosinate). The two 3HR lines were IRL (imidazolinone/glyphosate/ glufosinate).

The greenhouse dose-response experiment was conducted in the spring of 2002 at Saskatoon, Saskatchewan and repeated once. The factorial experiment was arranged in a completely randomized design with four replications (one pot per replicate). Four seeds were planted 1 cm deep in 10-cm square pots containing a mixture of soil, peat, vermiculite, and sand (3:2:2:2 by volume) plus a slow-release fertilizer (150 g of 26-13-0 per 75 L potting mixture). Experiments were conducted under a 20/16 C day/night temperature regime with a 16-h photoperiod supplemented with 230 µmol m^{-2} s^{-1} illumination. Pots were watered daily to field capacity. Four herbicide treatments were included in the experiment: 2,4-D amine, 2,4-D ester, MCPA ester, and metribuzin. Seedlings were treated at the two- to three-leaf stage. Herbicides were applied using a moving-nozzle cabinet sprayer equipped with a flat-fan nozzle calibrated to deliver 200 L ha^{-1} of spray solution at 210 kPa in a single pass over the foliage. Each herbicide was applied at 0.125, 0.25, 0.5, 0.75, 1, and 2 times the recommended rate plus an untreated control. The recommended rate in western Canada of 2,4-D amine, 2,4-D ester, and MCPA ester is 445 g ai ha^{-1}, whereas that of metribuzin is 215 g ai ha^{-1}. Commercial formulations of the herbicides were used. Twenty-one d after treatment, shoots were cut at soil level. Harvested biomass was dried at 80 C for 48 h, and weighed.

Response to 2,4-D at Two Growth Stages

The response of five canola cultivars at two growth stages to increasing doses of 2,4-D was examined in a factorial experiment. The greenhouse experiment was conducted during the winter/spring of 2002 and arranged in a randomized complete block design with four replications (one pot per replicate). The five canola cultivars used in the experiment were: (1) 'Quantum' (non-HR); (2) 'Conquest' (glyphosate HR); (3) 'InVigor 2663' (glufosinate HR); (4) '46A76' (imidazolinone HR); and (5) BX 263 (bromoxynil HR). The herbicide, 2,4-D amine, was applied at 70, 140, 280, 560, and 1,120 g ai ha^{-1} (plus an untreated control) to seedlings at the two- to three-leaf stage or the five- to six-leaf stage. Experimental conditions and procedures were similar to those described previously, except that fresh weight of shoot biomass was measured. Data were subjected to ANOVA. Statistical analysis of the dose-response curves followed the procedure detailed by Seefeldt et al. (1995).

Results and Discussion

Response to Alternative Herbicides

Data were combined across experiments upon confirmation of homogeneity of variances and lack of an experiment by treatment interaction. Response of shoot dry weight to increasing doses of the four herbicide treatments did not differ among canola cultivars/experimental lines (i.e., no canola by herbicide by dose interaction, $P>0.05$). Thus, all canola cultivars/lines responded similarly to increasing doses of each of the four herbicide treatments. A significant herbicide by dose interaction, however, was detected. The lack-of-fit F test indicated that the dose responses of the 11 cultivars/lines (pooled data) differed among metribuzin, 2,4-D, and MCPA (Fig. 1). No difference in dose response between the two 2,4-D formulations, however, was detected and thus data were combined. Metribuzin was most effective in controlling the 11 canola cultivars/lines, followed by MCPA then 2,4-D. All three herbicides, however, controlled the canola cultivars/lines well at a dose equivalent to the recommended rate.

Based on these results, canola with multiple herbicide resistance traits is not different than cultivars that are non-HR or single HR in their sensitivity to herbicides commonly used to control volunteers. Thus, the HR genes studied do not interact to produce an altered phenotypic (herbicide susceptibility) response. These findings agree with those of Senior et al. (2002) who found no difference in sensitivity among non-HR, glyphosate HR, glufosinate HR, and glyphosate plus glufosinate HR lines to paraquat (Group 22), metsulfuron (Group 2), and mecoprop (Group 4). Similarity in herbicide sensitivity suggests that these three herbicides can control canola volunteers that exhibit multiple herbicide resistance equally well as those that are non-HR or single HR.

Response to 2,4-D at Two Growth Stages

All five cultivars responded similarly to increasing doses of 2,4-D amine when treated at the two- to three-leaf stage (Fig. 2). Thus, these results agree those found in the previous experiment. A significant cultivar by growth stage by dose interaction ($P \leq 0.05$), however, indicated that the canola cultivars responded differently to increasing doses of 2,4-D at the five- to six-leaf stage compared with that at the two- to three-leaf stage. Efficacy of 2,4-D was markedly lower when plants were older and would result in poor control of volunteers under field conditions. Based on GR_{50} ratios, seedlings treated at the five- to six-leaf stage were at least 1.8-times more tolerant to the herbicide than those treated at the two- to three-leaf stage. The lack-of-fit F test indicated that the response of the bromoxynil HR cultivar to increasing doses of the herbicide differed from that of the other four cultivars (no difference in response among them). This cultivar was less sensitive to 2,4-D than the other cultivars. The reason for this differential sensitivity is unclear. We did not observe any apparent differences between these five cultivars in their response to increasing rates of 2,4-D applied at the two growth stages in a field experiment conducted in 2002 (unpubl. data), suggesting that the reduced sensitivity of the bromoxynil HR cultivar under controlled environment conditions may not be apparent under field conditions.

To effectively control canola volunteers, whether single HR or multiple HR, farmers should treat plants when they are most sensitive to herbicides (i.e., four-leaf stage or younger). Multiple HR *B. napus* volunteers that are beyond the four-leaf stage may be difficult to control (Beckie et al. 2001). Because volunteers can emerge early in the growing season and develop quickly, farmers may have a short period of time to treat them at this most sensitive growth stage. Adverse environmental conditions early in the growing season, especially cool temperatures, can impair growth and herbicide uptake into the plant and present another challenge for controlling volunteers.

There are over 30 registered herbicide treatments for control of single or multiple HR canola in cereals, the most frequent crop type to follow canola in a typical 4-yr rotation (Anonymous 2002). However, in some annual legumes, such as lentil or chickpea, or oilseed crops such as sunflower, there are few or no in-crop herbicide options. Therefore, complete field records and

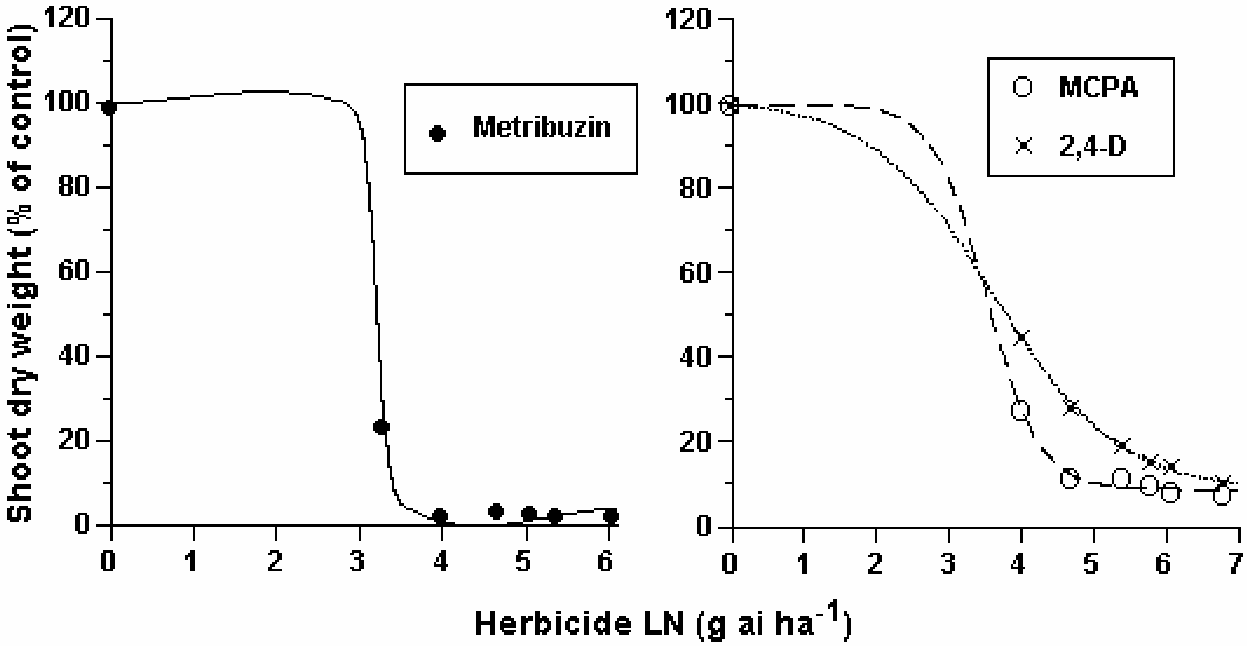

Figure 1. Response of 11 canola cultivars or experimental lines (pooled data) to increasing doses (natural logarithm, LN) of metribuzin, a Group 5 herbicide, and the Group 4 herbicides MCPA ester and 2,4-D (amine and ester data are pooled).

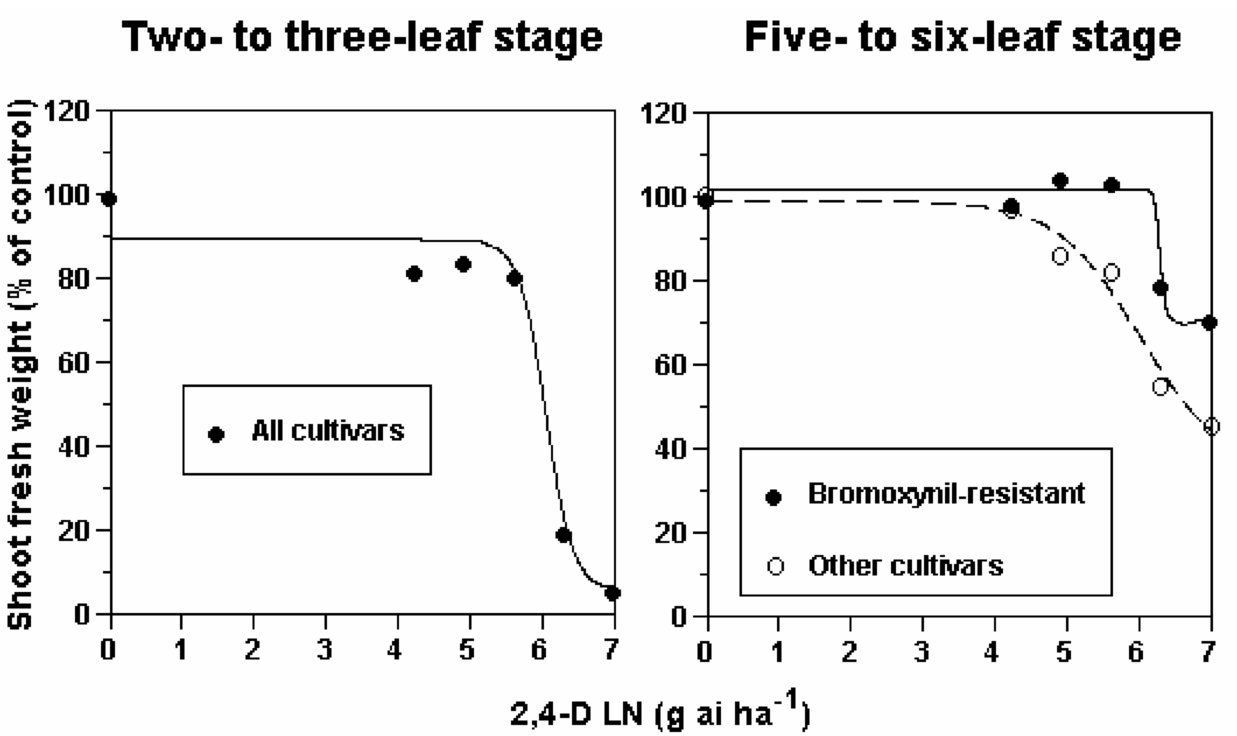

Figure 2. Response of five canola cultivars [glyphosate herbicide-resistant (HR), glufosinate HR, imidazolinone HR, bromoxynil HR, and non-HR] to increasing doses (natural logarithm, LN) of 2,4-D amine at the two- to three-leaf stage (all cultivars pooled) and five- to six-leaf stage (bromoxynil HR vs. other four cultivars).

careful crop rotation planning are required when these and some other broadleaf crops are grown.

Acknowledgments

This study was supported by the Canadian Biotechnology Strategy Program, Government of Canada (Project 1999-14-002). We acknowledge the excellent technical expertise of Christopher Lozinski and Herb Schell.

Anonymous. 2002. Guide to Crop Protection 2002: Weeds, Plant Diseases, Insects. Bi-provincial publication, Regina, SK: Saskatchewan Agriculture and Food; Winnipeg, MB: Manitoba Agriculture and Food. 354 p.

Beckie, H. J., Hall, L. M. and Warwick, S. I. 2001. Impact of herbicide-resistant crops as weeds in Canada. Proceedings, Brighton Crop Prot. Conf. – Weeds. Farnham, Surrey, UK: British Crop Protection Council. pp. 135-142.

Gulden, R. H., Shirtliffe, S. J. and Thomas, A. G. 2002. Evidence of cyclical dormancy behaviour in spring *B. napus*. Pages 41-44 *in* D. Bernier, R. A. Campbell, and D. Cloutier, eds. Proceedings of the 2001 National Meeting of the Expert Committee on Weeds/Comité d'experts en malherbologie, Québec City, Québec, Canada. Sainte-Anne-de-Bellevue, QC: ECW-CEM.

Gulden, R. H., Shirtliffe, S. J. and Thomas, A. G. 2003. Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. Weed Sci. **51**:(*in press*).

Hall, L., Topinka, K., Huffman, J., Davis, L. and Good, A. 2000. Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. Weed Sci. **48**:688-694.

Légère, A., Simard, M. J., Thomas, A. G., Pageau, D., Lajeunesse, J., Warwick, S. I. and Derksen, D. A. 2001. Presence and persistence of volunteer canola in Canadian cropping systems. Brighton Crop Prot. Conf. B Weeds. Farnham, Surrey, UK: British Crop Protection Council. pp. 143-148.

Pekrun, C., Hewitt, J. D. J. and Lutman, P. J. W. 1998. Cultural control of volunteer oilseed rape (*Brassica napus*). J. Agric. Sci. **130**:155-163.

Seefeldt, S. S., Jensen, J. E. and Fuerst, E. P. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. **9**:218-227.

Senior, I. J., Moyes, C. and Dale, P. J. 2002. Herbicide sensitivity of transgenic multiple herbicide-tolerant oilseed rape. Pest Manag. Sci. **58**:405-412.

Thomas, P. 2001. You can control multiple-resistant canola volunteers. Canola Guide, April 2001 issue. Winnipeg, Manitoba: Farm Business Communications [www.agcanada.com]. pp. 48-50.