3º PAINEL – INTEGRAÇÃO HERBICIDAS X AMBIENTE

ENVIRONMENTAL IMPACT OF HERBICIDE-RESISTANT *BRASSICA NAPUS* (CANOLA) IN CANADA

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Herbicide resistant *Brassica napus* has been widely adopted since its introduction in 1996. It was sown on over 80% of the canola area cropped in western Canada in 2002. Potential environmental risks have been examined, including herbicide resistant gene movement between species and between types of canola, and the effect of modified herbicide use patterns and agronomic practices. Cruciferous weeds, *B. rapa*, *Raphanus raphanistrum, Erucastrum gallicum* and *B. kaber,* with some potential to introgress, co-exist in or adjacent to *B. napus* crops. Screening has not yet detected herbicide resistant weed biotypes conferred by introgression. However, herbicide use has already selected many resistant and multiple herbicide resistant weed species in western Canada. The risk of introgression of herbicide tolerant genes into weedy biotypes must be viewed in relation to risk of natural selection. Depending on the species, weed density and herbicide, risk of natural selection may be higher than from introgression. *B. napus* has approximately 20% out-crossing rates and volunteer multiple herbicide resistant *B. napus* due to gene movement via pollen have been identified in farm fields. Control of volunteer *B. napus* prior to and in subsequent crops has therefore become more complex. However, total herbicide use has declined in herbicide resistant canola, an estimated reduction of 6,000 tonnes in each of 1999 and 2000, with a consequent reduction of 40% in herbicide costs. Reduced tillage for weed control and incorporation of soil-applied herbicides in herbicide resistant *B. napus*, resulted in 31.2 million liters less fuel used in 2000. Additionally, reduced tillage decreased soil degradation and erosional losses. Farm profits are greater in herbicide resistant canola, by an average of $14.32 (Canadian) per ha. On balance, it appears that herbicide resistant canola has the potential to enhance the environmental and economical sustainability of *B. napus* production

Introduction

In western Canada, the major canola growing region in Canada, 75,000 growers produced an average of 4.7 million ha of canola over the past three years. Two species, *B. rapa and B. napus*, were bred and are used

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commercially to produce canola quality oil, but, because of the higher yield of *B. napus,* that species is grown on approximately 90% of canola acreage. Four types of herbicide resistant *B. napus* are currently grown (Table 1) and make up over 80% of the *B. napus* grown in western Canada in 2002 (M. Hartman, personal communication). Herbicide resistance to glyphosate, glufosinate and bromoxynil was conferred by recombinant DNA technologies while resistance to imidazolinone herbicides was selected following mutagenesis. In Canada, all herbicide resistant canola types are regulated as plants with novel traits (PNT's). They are regulated by the Canadian Food Inspection Agency (CFIA) who state "plants in Canada are regulated on the basis of the traits expressed and not the basis of the method used to introduce the trait. PNTs may be produced by conventional breeding, mutagenesis or recombinant DNA techniques." International concern, however, has focused on plants containing transgenes.
Producers have rapidly and readily adopted herbicide resistant technologies in canola (Canola Council of Canada). This paper will to outline the impact of these new technologies on the environment in Canada.

Super Weeds – introgression of herbicide resistance into weedy or native taxa

Brassicae, like most domestic taxa, mate with wild or weedy relatives and have a potential to influence the evolution of wild plants (Ellstrand et al. 1999). Introgression of transgenes (the stable incorporation of a gene into the genome) and the creation of 'super weeds' are frequently raised as a concern in the public forum.
Brassica napus **contains an A genome from** ***B. rapa*** **and the C genome from** ***B. oleracea. B. rapa*****, one of the parental species, is bred as a canola crop in Canada, and is a widely distributed weedy species in many parts of the world (reviewed in Warwick et al. 2000).** ***B. napus*** **and** ***B. rapa*** **spontaneously hybridize. Jørgensen and Andersen (1994) found** ***B. napus*****-specific allozyme alleles in two wild** ***B. rapa*****-like plants in Denmark - evidence of past hybridization and introgression.**
B. napus **co-exists in Canada with several weedy relatives with potential to cross pollinate, including wild radish (*****Raphanus raphanistrum)*****, dog mustard (*****Erucastrum gallicum*****), and wild mustard (*****B. kaber*****) (Warwick et al. 2000). The potential for hybridization and introgression depends on a myriad of factors, including species, hybridization frequency, distribution and abundance of weeds, co-incidence of flowering time, fitness of hybrids and subsequent selection by herbicides. The interactions of these factors generally reduce the likelihood of introgression. Surveys are currently being conducted in Canada to assess the presence of transgenes in weeds,**

including *B. rapa*. However rare, the scale of potential pollen flow and the evolutionary time frame allow us to predict these events may occur.

Super weeds – existing herbicide resistant weeds in western Canada

Western Canadian agronomic practices have selected for a wide range of herbicide resistant weeds, including several broadleaf weeds resistant to ALS inhibitors (Beckie et al. 2000a) and green foxtail (*Setaria viridis*) resistant to DNA herbicides (Table 2). Currently in Western Canada there are over 10 herbicide resistant and multiple resistant weed species (International Survey of Herbicide Resistant Weeds, 2002) including wild oat (*Avena fatua*) resistant to herbicides in four distinct herbicide mode of actions (ACCase inhibitors, ALS inhibitors, VLC fatty acid biosynthesis inhibitors, and flamprop-methyl with an unknown mode of action). Management of herbicide resistant weeds has relied on herbicide mixtures, herbicides with alternative modes of action and non-chemical methods. For producers with these weeds, cropping options are limited, and weed control becomes the focus of agronomic practice.

Herbicide rotation options, within conventional canola, are limited. Herbicide tolerant canola provides growers with additional herbicide options. For example, wild oat can be controlled by glyphosate or glufosinate in tolerant canola, and wild oat resistant to these products has not been reported any where in the world.

The proclivity of an herbicide to select for herbicide resistance depends primarily on its mode of action (Beckie et al. 2000a). For example, ALS inhibitors and ACCase inhibitors select rapidly for herbicide resistance. Herbicide resistant canola has provided growers with the use of two new herbicide groups, glyphosate and glufosinate which are less prone to select for resistance. Their use will delay the selection of additional weeds resistant to conventional herbicides, while offering chemical options for control of existing resistant species.

Volunteer canola as a weed

Volunteer canola (both *B. napus* and *B. rapa*) is one of the more common weeds in subsequent crops in Western Canada. In a recent survey in Alberta, volunteer canola ranked as the 16th most abundant weed in all crops (Leeson et al. 2002). It should be noted that because of the difficulties in differentiating volunteer from planted canola, volunteer canola in canola crops is underestimated in weed surveys. The frequency of volunteer canola decreases with time after the initial crop (Légère et al. 2001). However four years after planting canola, volunteer canola was still present at low densities of 0.2 to 0.5 plants m^{-2}. The presence of volunteer canola extends the potential for cross-pollination with other herbicide resistant canola varieties and introgression with weedy species spatially and temporally.

Herbicide resistant and conventional *B. napus* can all be controlled by auxinic herbicides in most subsequent crops (Beckie et al. 2001b). In some legume crops, auxinics cannot be used and control is limited to non-chemical means. Glyphosate resistant volunteers cannot be controlled by glyphosate applied alone pre-seeding or in chemical fallow. Should more glyphosate resistant crops become available, weed control will become more complex. Imidazolinone-resistant canola cannot be controlled by ALS inhibitors alone in foliar applications. Glufosinate resistant canola does not restrict subsequent glufosinate use, as this herbicide is not currently used in other crops.

Gene flow between canola crops

B. napus is a partially outcrossing species, with plant to plant outcrossing rates of approximately 20% (Rakow and Woods 1987). When herbicide resistant varieties were released, it was predicted that cross pollination would occur and these volunteers might be resistant to more than one type of herbicide. However, the extent and distance of potential pollen flow was not well defined.

During an investigation of the causes of unexpected herbicide resistance in a field in Alberta, it was determined that glyphosate resistant volunteers could be found over 500 meters away from the pollen source and that these volunteers, having continued to propagate could have seed resistant to three different types of herbicides (Hall et al. 2001). Further work conducted in Western Canada (Beckie et al. 2001b) and in Australia (Reiger et al. 2002) has confirmed *B. napus* genes can move long distances with pollen and that frequency of outcrossing is not easily correlated with distance. Frequency did not diminish linearly with distance, nor was a low frequency maintained consistently over a long distance.

Pollen flow between canola fields, in conjunction with long term volunteer survival suggests that gene flow may be extensive. Unlike introgression and the formation of 'super weeds', there is ample evidence that multiple resistant canola volunteers are present in measurable frequencies and would be very difficult to eradicate within a canola field. This is not a concern within fields destined for commercial production; however such volunteers are not acceptable when certified seed production or organic certification is required.

Agronomic practices in conventional and herbicide resistant canola

Prior to the release of herbicide resistant *B. napus*, weed control in canola was perceived by growers as the primary factor limiting canola yield and acreage. Few herbicides could be safely applied (Table 1).

Soil applied dinitroaniline herbicides necessitated tillage for incorporation and their use in spring delayed seeding, thus reducing yield potential. The

required tillage slowed the adoption of soil conservation practices, thereby increasing soil erosion and the loss of organic matter. DNA's had a limited weed spectrum and efficacy was subject to environmental influences. These herbicides are applied at relatively high rates, and have relatively long residual activity in the soil. They are not toxic to humans but are toxic to fish.
Broadleaf herbicide options (Table 1) in conventional canola were limited because of crop tolerance, and those available, clopyralid and ethametsulfuron methyl had a narrow weed spectra. Weed control necessitated a three year crop rotation prior to canola. Both products are non-toxic, but clopyralid has higher use rates and a longer half life in soil. Grass herbicides used in canola are moderately toxic, but most have a short residual time and relatively low use rates.

The introduction of herbicide tolerant canola has allowed for greater sustainability, in terms of greater weed control, more environmentally friendly agronomic practices, and a decrease in herbicide usage and costs. Total herbicide use in canola was estimated to decrease by 6,000 tonnes in each of 1999 and 2000 (Devine and Buth 2001) because of herbicide resistant canola.

The introduction of herbicide tolerant canola facilitated producers adopting direct seeding. Producers who grow herbicide resistant canola are more likely to use conservation practices than producers who grow conventional canola (Canola Council of Canada 2001). The benefits of reducing soil and water loss, and subsequent degradation of soil fertility contribute to environmental sustainability.

Reductions in the use of tillage for weed control and incorporation of soil-applied herbicides in herbicide resistant *B. napus*, resulted in an estimated reduction of 31.2 million liters less fuel used by farm machinery in 2000. On balance, herbicide tolerant canola increased grower return per ha by $14.32.

A recent extensive survey identified producers' rationale for growing herbicide tolerant canola (Canola Council of Canada). They stated the following reasons (multiple responses were given):

- **50% wanted easier and better weed control**
- **19% anticipated a better yield and better return and more profit**
- **18% expected better grassy weed control**
- **15% expected better broadleaf weed control**
- **10% did so to reduce costs**
- **9% did so to compare varieties**
- **7% did so to clean up their fields**
- **7 % wanted to reduced the number of herbicide applications**
- **5% wanted better perennial broadleaf weed control**
- **3% wanted to reduce tillage**
- **3% did it for herbicide rotation**

- **2% wanted to be able to seed earlier and save soil moisture**

Conclusions

The balance between ecological gains and losses must account for the probability of occurrence and the ecological consequences of both current and new practices. The low risk of introgression and creation of 'super weeds' should be balanced with the current reality of multiple herbicide resistant weeds. The potential for gene flow between crops and volunteers must be weighed against the gains in sustainable practices and reduced fuel and herbicide usage. Finally, the sustainability of farming practices must encompass economic sustainability and the viability of an agricultural industry.

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Table 1. Herbicide-resistant *B. napus* currently grown in Western Canada

Initiated	Herbicide	Resistance	Seed Protection	Control Measures	Market* (%)
1995	glufosinate	*pat* or *bar* transgene	Some hybrid varieties	Like conventional canola	15
1996	glyphosate	*epsps* + *gox* transgenes	Contracts prohibited seed use	Chem-fallow and pre-seeding treatments require modification	55
1996	imidazolinones	ALS modified via mutagenesis	Growers free to re-seed	Cannot use ALS inhibitors alone in-crop	15
2000	bromoxynil	nitrilase transgene	Growers free to re-seed	Like conventional canola	<1

***2002, estimated (M. Hartman, personal communication)**

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Table 2. Herbicides used on conventional and herbicide resistant canola. Environmental impact is a function of the amount of product applied, the half life of the product in soil and oral toxicity.

Product	Mode of Action	Spectrum	Resistant Weeds Present	Use Rate (gai ha^{-1}) *	Average Half - life in Soil * (days)	Toxicity (Oral LD_{50}) *
Conventional Canola Production						
Trifluralin	Mitotic Inhibitor	Grass and some broadleaf control	Yes	560-830	45	>5000
Ethalfluralin	Mitotic Inhibitor	Grass and some broadleaf control	Yes	845-1380	60	>5000
Metsulfuron methyl	ALS inhibitor	Limited broadleaf weed control	One	15-22	20	>5000
Clopyralid	Auxinic herbicide	Primarily Canada thistle control	None	150-200	40	4300