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Biosafety Assessment of Plants with Novel Traits in Canada

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ABSTRACT

Canada has a unique system for regulating products of agriculture biotechnology, based upon familiarity, substantial equivalence, and trait novelty. Plants with novel traits (PNTs) are regulated; such plants can be transgenic or non-transgenic. Safety assessments are required before a PNT can be used as a food, livestock feed, or released unconfined into the environment. This paper outlines the regulation of PNTs, environmental safety assessments of PNTs, and a case assessment study of herbicide-resistant *Brassica napus* canola in Canada. Since 2005, 62 PNTs have been authorized for environmental release, possessing such traits as herbicide resistance, insect pest and virus resistance, and oil compositional changes. Future challenges in regulation include the interpretation of novelty, new classes of PNTs (e.g., stress tolerance, molecular farming, biofuels production), and evaluating long-term and non-target effects.

Key words: *Brassica napus*; Biosafety risk assessment; Transgenic crop.

INTRODUCTION

Canada is the fourth largest producer of transgenic crops, after the United States, Argentina, and Brazil (Beckie *et al.* 2006). Canada regulates plants with novel traits (PNTs). A PNT is defined as "a plant containing a trait not present in plants of the same species already existing as stable, cultivated populations in Canada, or is present at a level significantly outside the range of that trait in stable, cultivated populations of that plant species in Canada. PNTs may be produced by conventional breeding, mutagenesis, or more commonly, by recombinant DNA (rDNA) techniques" (CFIA 2004). The primary assumption underlying this approach is that there are similar environmental risk issues regardless of the process of developing a PNT. Therefore, in contrast to other countries, Canada regulates a broader range of plants, including those with traits selected following mutagenesis or introgressed following outcrossing. There are two main concerns by industry or breeders of this regulatory approach. A plant not produced by rDNA technology is subject to regulation, whereas the same plant in other countries is not. Secondly, products requiring regulation are not as easily identified as with a process designation (i.e., products produced using rDNA techniques), causing uncertainties among cultivar developers.

Canada's regulatory approach is based on the principles of familiarity (familiar counterpart as a comparator), substantial equivalence (defines the threshold of risk), and trait novelty. 'Novel' does not necessarily equate with 'risk'. A product may be considered novel if it: (1) possesses a new trait(s) or characteristic(s) – e.g., herbicide resistance, insect resistance; (2) possesses a modified trait(s) or characteristic(s) – e.g., disease resistance outside the range of normal variation within that species; or (3) is considered for a new use (e.g., as a food or livestock feed). Most products of breeding programs would not be considered PNTs. Similarly, many products of mutagenesis may not be considered PNTs (e.g., trait changes within normal ranges). Risk assessment focuses on unintended, not unexpected effects. This paper outlines the regulation of PNTs, environmental safety assessments of PNTs, and a case assessment study of herbicide-resistant (HR) Brassica napus canola in Canada.

Regulation of Plants with Novel Traits in Canada

Mandatory, pre-market safety assessments are required by government regulators before a PNT can be used as a food, livestock feed, or released unconfined into the environment. Therefore, PNTS are assessed based on potential impact on the environment, potential impact on livestock health, and potential impact on human health. The Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) is responsible for the regulation of the environmental release of PNTs and feed assessments (Fig. 1). CFIA responsibilities include approval and inspection of confined field trials of PNTs, approval of unconfined release of PNTs, and assessment of import applications for PNTs. Health Canada conducts food safety assessments. Food, feed, and environmental release authorizations are linked; all three are required before a PNT is approved.

The key element of the Canadian federal regulatory framework for PNTs is to use existing legislation and regulatory institutions; there is no “Gene Act” or “Biotechnology Agency” (Fig. 1). Fig. 2 outlines the regulatory approval process for PNTs. The main concepts for the regulation of PNTs are: (1) science-based and product-based; (2) case-by-case; (3) regulatory transparency (decision documents and consultations available online); (4) harmonized approvals between Canadian regulators and bilateral initiatives with the United States (i.e., data requirements); and

(5) research in support of the regulatory system.

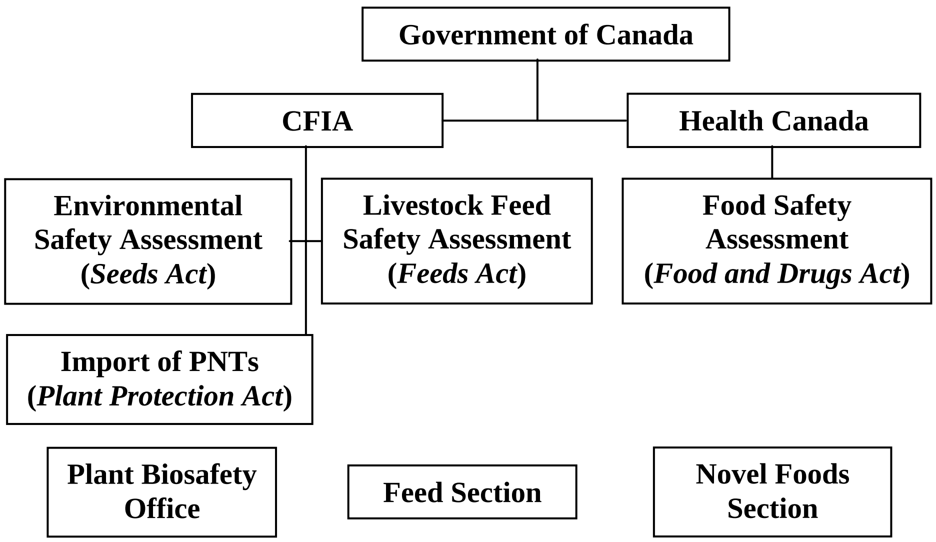

Fig. 1. Regulation of plants with novel traits in Canada using existing legislation.

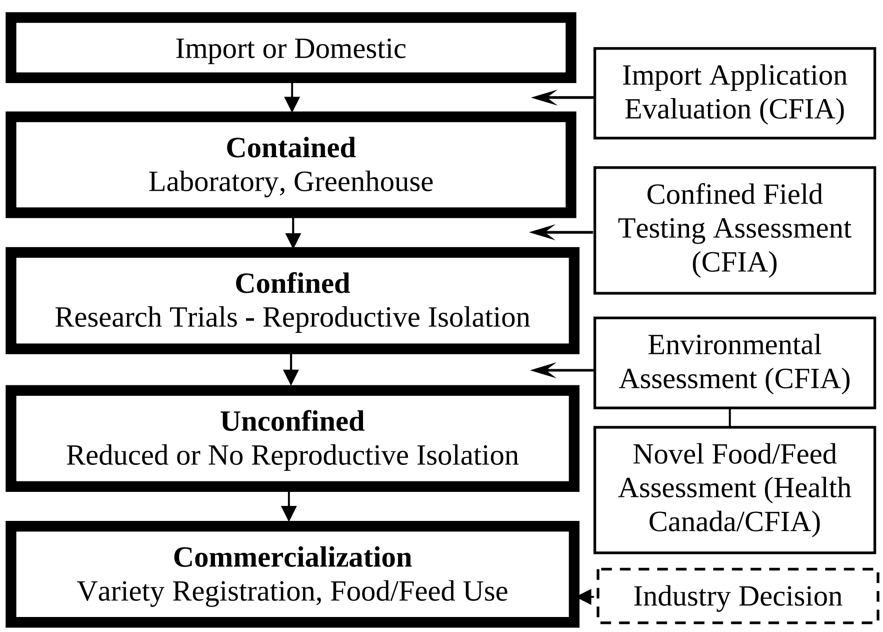

Fig. 2. Regulatory approval process for plants with novel traits in Canada.

Approvals for unconfined environmental release of PNTs have been granted since 1995. Since 2005, 62 PNTs (52 of which are transgenic) have been authorized for environmental release. These PNTs include canola (oilseed rape, *Brassica napus*; turnip rape, *B. rapa*), corn, soybean, wheat, cotton, flax, lentil, potato, soybean, squash, sugar beet, and tomato. Traits include herbicide resistance; insect pest and virus resistance; hybrid systems; and oil compositional changes. The actual release is a commercial decision. The time to commercialize a PNT in Canada has been 8 to 10 years (Beckie *et al.* 2006).

Table 1. Field crops with novel traits grown commercially in Canada since 1995 (NA, not required; adapted from Beckie et al. 2006).

Species	Herbicide resistance	Variety registration	Regulatory approval	Breeding system
Canola, *Brassica napus*	Glyphosate	Yes	Yes	ca. 30% outcrossing
	Glufosinate	Yes	Yes	
	Imidazolinone	Yes	Yes	
	Bromoxynil	Yes	Yes	
Soybean, *Glycine max*	Glyphosate	Yes	Yes	Highly selfing
	Glufosinate	No	Yes	
Corn, *Zea mays*	Glyphosate	NA	Yes	Highly outcrossing
	Glufosinate	NA	Yes	
	Imidazolinone	NA	Yes	
	Sethoxydim	NA	Yes	
Wheat, *Triticum aestivum*	Imidazolinone	Yes	Yes	Highly selfing
Lentil, *Lens culinaris*	Imidazolinone	Yes	Yes	Highly selfing

The first approved PNTs possessed enhanced input traits, mainly herbicide resistance, that enabled them to tolerate the application of selective (imidazolinones, IMIs; bromoxynil, BX) or non-selective (glyphosate, GLY; glufosinate, GLU) herbicides. The GLY-, GLU-, and BX-HR crops in Canada were produced by rDNA techniques through the insertion of bacterial genes (i.e., transgenic), whereas IMI resistance was attained by chemical mutagenesis (i.e., non-transgenic) (CFIA 1995a, 1995b, 1995c, 2004). Five field crops are currently grown commercially in Canada (Table 1). Corn hybrids may have stacked traits (i.e., HR and *Bt*). Canola and spring wheat are grown predominantly in western Canada, whereas soybean and corn are grown mostly in eastern Canada. Canola occupies over 80% of the area cultivated to novel-trait crops, followed by soybean, corn, and wheat in order of decreasing area (Beckie *et al.* 2006).

Environmental Safety Assessments

PNTs may be granted approval for confined environmental release (i.e., field research trials), which may be followed by unconfined environmental (commercial) release. Confined environmental release comprises risk mitigation measures, plus inspection and monitoring. All confined field trials are restricted to a maximum of 1 ha per site, maximum of 10 sites per province, with a cumulative size of no more than 5 ha per province. Reproductive isolation distances are the following: *Brassica rapa* canola, 400 m; *Brassica napus* canola, 200 m; corn, 200 m; soybean, 10 m; wheat, 30 m; and potato, 1 m. Other options to reduce pollen flow include cages, nets, or bags, guard/border rows, or flower removal. The applicant is responsible for monitoring the trial site on a regular basis and for keeping the trial in compliance. The applicant must keep monitoring records. CFIA inspects all sites, unannounced, for compliance to the terms and conditions. All plant materials must not enter the food or feed chain without federal government approval must be stored securely if not disposed of, and may be disposed of in a manner which destroys viability. Post-harvest restrictions and monitoring include trial plots not planted to the same crop for a specified period of time (e.g., 3 years for *Brassica napus* canola, 1 year for corn), and each site must be monitored during the post-harvest growing seasons for volunteers and related/prohibited species. Applications for confined field trials peaked in 1997 (Figure 3). Figure 4 shows trait trends in such trials.

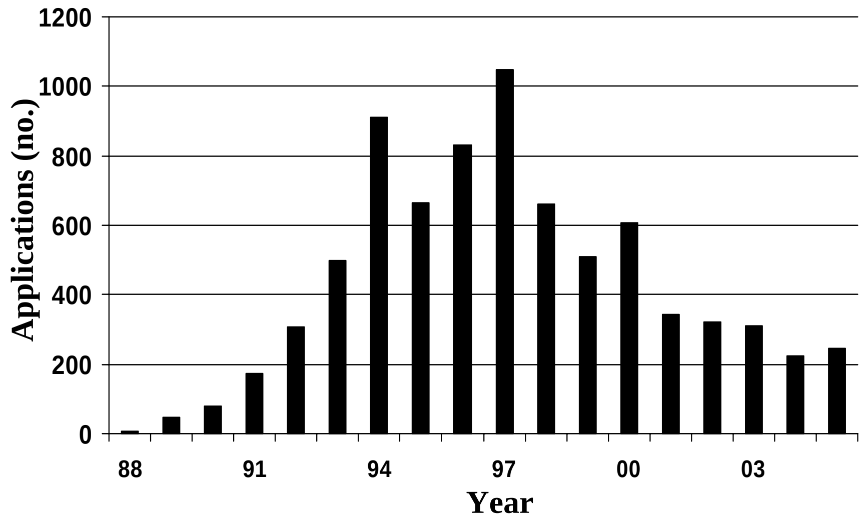

Fig. 3. Number of applications for confined field trials of PNTs in Canada.

Criteria for unconfined environmental release of a PNT includes: (1) potential to become a weed of agriculture or be invasive of natural habitats; (2) potential for gene flow to wild relatives whose hybrid offspring may become more weedy or more invasive; (3) potential to become a plant pest; (4) potential impact (including its gene products) on non-target species, including humans; and (5) potential impact on biodiversity. The applicant must address the identity and origin of the PNT; the properties of the novel gene and gene products; the relative phenotypic expression of the PNT compared to a similar counterpart, where differences are anticipated; and anticipated or known relative effects on the environment resulting from the release. Information regarding the PNT must include a description of the PNT; description of the modification of the PNT; description of the inheritance and stability of introduced traits functional in the plant; description of parental genome; number of generations removed from the original modification; and description of novel traits and how they were introduced. Information required for unconfined release assessment also includes the biology, ecology, and interactions of a PNT. Such information includes description of the biology of the plant species prior to modification; selection of a suitable counterpart; phenotype of the PNT; cultivation of the PNT; interactions with sexually-compatible species; residual effects and toxicity on non-target organisms; and other environmental interactions.

Information is needed to answer key questions, such as have you created a weed?; what are the consequences of gene flow?; does it cause disease in plants?; does it have unintended effects on non-target organisms? For intra-specific crosses, once a PNT is authorized, all its progeny and sister lines which have been derived from the original transformation and their respective progenies are also authorized for unconfined release provided the applicant has determined that no inter-specific crosses were performed, the intended uses are similar, and these plants do not display any additional novel traits. For inter-specific crosses, once a PNT is authorized, plants from the first inter-specific cross may require an environmental safety assessment unless the applicant has determined there is no new transformation event; the intended uses are similar; these plants do not display any additional novel traits based on their characterization; and the novel genes are expressed at a level similar to that of the authorized line. For intentional trait stacking, the applicant must contact the PBO 60 days prior to the anticipated environmental release. The PBO may request and review data to support the safe use of the modified plant in the environment. Stacking of approved traits with potential incompatible management requirements, possible negative synergistic effects, or where production of the plant may be extended to a new area of the country may elicit an environmental safety assessment.

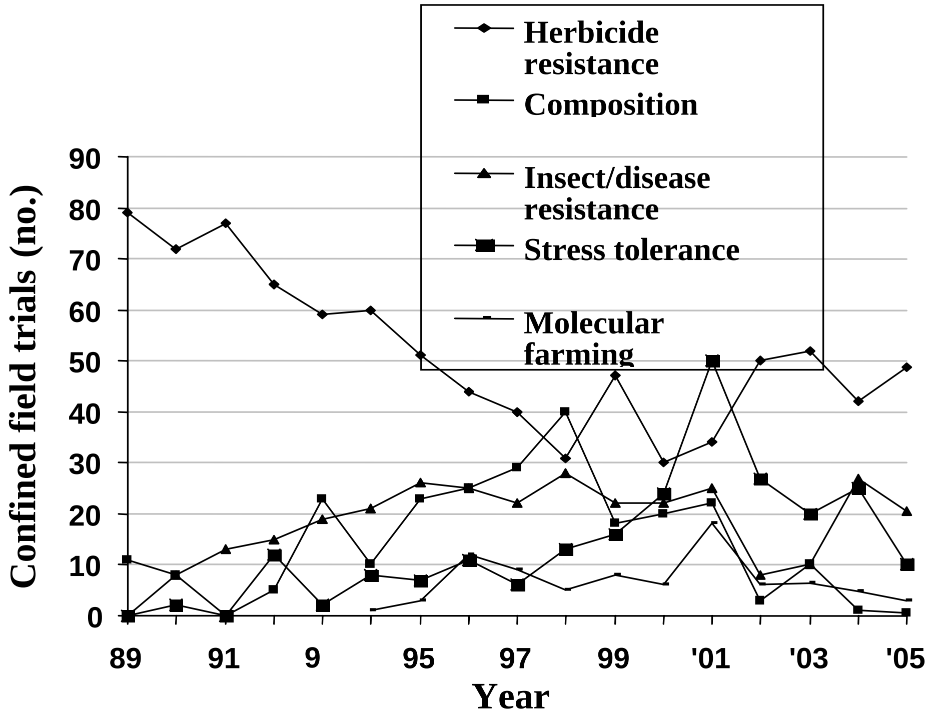

Fig. 4. Types of traits in confined field trials in Canada.

The applicant is required to outline stewardship plans (e.g., insect or herbicide resistance management, post-release monitoring). For herbicide resistance management, the applicant should address control of volunteers, changes in usual agronomic practices that may arise and result in reduced sustainability or have significant impacts on soil conservation; selection of herbicide resistance in weeds resulting from the potential continued application of the same herbicide mode of action in subsequent rotations; introgression of novel trait into related species; management of the HR crop during the growing season, particularly where multiple herbicide resistance due to cross-pollination could arise in volunteers in subsequent growing seasons; communication to growers as well as an efficient mechanism allowing growers to report problems to the developer; and monitoring the effectiveness of the stewardship plan. Companies are required to report the results of their own monitoring activities to CFIA.

CASE STUDY

Biosafety Assessment of Herbicide-resistant *Brassica napus* Canola

Canola is grown primarily as an oilseed crop; after the oil is extracted, the meal is used as a livestock feed. Because significant populations of weedy bird's rape (*Brassica rapa*) is found in eastern Canada, glyphosate is used to control the weed, and outcrossing with *B. napus* canola occurs, approval of glyphosate-resistant *B. rapa* canola was restricted to western Canada. The PNT is no longer commercialized in Canada.

Sustainability underlines the decision-making process; the introduction of a new HR crop should have at least a neutral impact. In these environmental assessments (CFIA 1995a, 1995b, 1995c), the key environmental question was what advantage would be conferred outside of managed environments in the absence of selection by the specific herbicide? HR crops are not more fit; transfer of herbicide resistance does not confer fitness outside of managed environments (Beckie *et al.* 2006). What are the consequences of gene flow - e.g., the loss of the use of the herbicide for control of crop volunteers and possibly for related weeds? Other questions to address: are there suitable management options for volunteers and how does the new trait affect current crop management practices by both adopters and non-adopters?; are growers able to use the same crop management practices? will herbicide selection pressure for evolution of HR weeds increase significantly? Agronomic problems can become environmental problems. For HR canola, growers are using similar crop management techniques and similar rotations (Beckie *et al.* 2006). Gene stacking occurs in canola volunteers; this was acknowledged in the decision documents. However, such volunteers are not more difficult to control than non- or single-HR plants and can be managed without increasing herbicide use or tillage (Beckie *et al.* 2006); integrated weed management (e.g., crop and herbicide rotation) is essential in HR canola production systems to mitigate herbicide resistance in weeds (Beckie 2006). To this end, growers must be made aware of proper herbicide resistance management practices and tactics.

FUTURE CHALLENGES

Future challenges in the regulation of PNTs are (1) to develop clear guidance on the interpretation of novelty that can be applied to all types of plants, e.g., crops, ornamental plants, and trees; (2) develop guidelines for molecular farming; (3) regulatory streamlining (import vs. domestic, PNTs vs. novel plants); (4) how to evaluate long-term and non-target effects (e.g., suitable indicator species, acceptable protocols); and (5) how to regulate new classes of PNTs, e.g., canola grown for biodiesel.

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